Diet, spatial ecology and energetics of echidnas: the significance of habitat and seasonal variation

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The research associated with this thesis abides by the international and Australian codes on human and animal experimentation, the guidelines by the Australian Government's Office of the Gene Technology Regulator and the rulings of the Safety, Ethics and Institutional Biosafety Committees of the University.

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Statement of publication and co-authorship

The following people and institutions contributed to the publication of the work undertaken as part of this thesis:

Paper 1:
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Candidate (50%), author 1 (Niels Andersen) (25%), author 2 (Stewart Nicol)(25%)

Details of the Authors roles:
Authors 1 and 2 contributed to the concept and planning of the study, and to data collection. Author 2 assisted with data analysis.

We the undersigned agree with the above stated "proportion of work undertaken" for the above published peer-reviewed manuscript contributing to this thesis:

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The following people contributed to the research undertaken as part of this thesis

Stewart Nicol (School of Zoology, University of Tasmania) provided guidance on data collection and assistance with statistical analysis and provided constructive criticism of data analysis, interpretation of results and chapter drafts.

Sue Jones (School of Zoology, University of Tasmania) supervised my radioimmunoassay work (Chapter 2) and provided constructive criticism of data analysis, interpretation of results and drafts of all chapters.

Niels Andersen (School of Medicine, University of Tasmania) provided field assistance and guidance on data collection (Chapters 2, 3, 4 and 5) and constructive criticism of data analysis, interpretation of results and chapter drafts (Chapters 5a).

Rob Gasperini (Menzies Research Institute) performed western blot analysis of leptin (Chapter 2)

Craig Mundy (TAFI, University of Tasmania) calculated home range estimates in R and converted them into a format suitable for use in a GIS (Chapter 4).

Mark Hovenden and Mike Perring (School of Plant Science, University of Tasmania) calculated pasture productivity at Lovely Banks (Chapter 6).

This thesis is based on field data collected by me in the period of August 2004 to October 2010, but also included data collected as part of a long term project of echidna field biology which was started in 1997. A small number of additional data collected by other researchers has been included in the data analysis for **Chapter 2 -** Seasonal variation in circulating leptin concentrations and body mass in free ranging short beaked echidnas (*Tachyglossus aculeatus*) and **Chapter 4 -** The influence of habitat type on the home range size of the short beaked echidna (*Tachyglossus aculeatus*).

Abstract

The ant- and termite-eating echidna is a solitary, monotreme mammal with a highly seasonal life history. I investigated several aspects of the seasonal energetics and ecology of free-ranging echidnas: the role of leptin in the annual mass cycle, seasonal and habitat effects on diet, the relationship between home range size and habitat quality, and the effect of habitat on the siting of echidna latrines.

The echidna has a large seasonal variation in fat stores, which reach their maximum prior to hibernation. I hypothesised that the hormone leptin would have the same role in the echidna as in eutherian hibernators, i.e. that there would be a direct relationship between body mass and plasma leptin that would change to allow pre-hibernatory fattening. I found significant seasonal variations in plasma leptin, with the highest levels occurring in hibernation and in females during mating. The lowest levels were found in males after the reproductive period. Rather than the expected strong positive relationship between adiposity and plasma leptin I found a weak negative relationship, similar to that in reptiles and birds.

To determine if there was any significant seasonal variation in diet associated with pre-hibernatory fattening I investigated diet using scat and stable isotope analysis. Echidna scats consisted largely of ants and the larvae of pasture cockchafer beetles. There was significant seasonal variation in percent occurrence of larvae, but not in the ant species found in scats or in the stable isotopic composition of echidna plasma. Although there was no difference in the prey items that contributed most to scat contents of animals living in different habitats, stable isotope analysis of blood showed a highly significant effect of habitat type on $\delta^{15}N$.

Female echidnas showed a significant negative relationship between the proportion of woodland habitat and home range size, whereas there was no such relationship for males, which had significantly larger home ranges. My data suggest that female home range is scaled to available resources, while male home range is probably scaled to maximise access to females.

The role of latrines and their relationship to habitat was examined by a detailed survey of part of the field site, where many echidna home ranges were known to overlap. Latrines were located more frequently in scrub than in pasture or thick bush and the highest frequency of latrines occurred where there were two home ranges that overlapped. Latrines may be important in the spatial organisation of echidnas.

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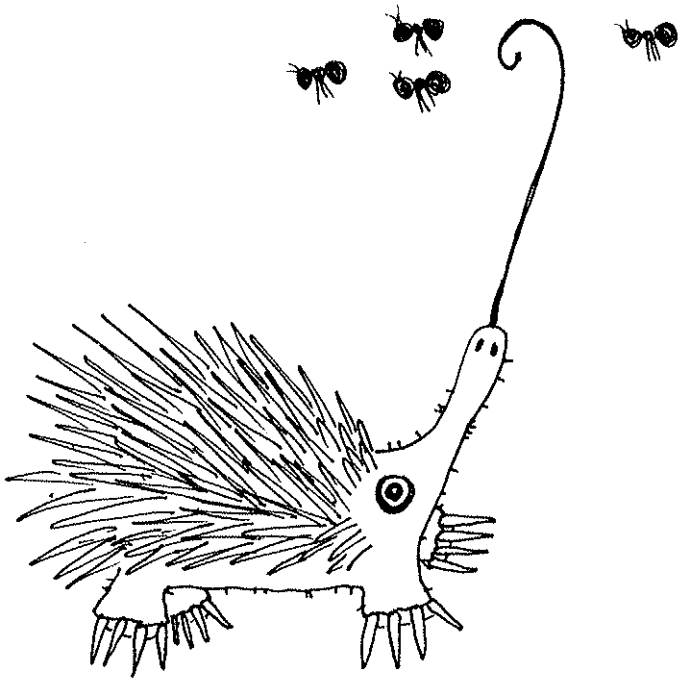

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Table of Contents

Chapter 1

General Introduction

General Introduction

Environmental variation shapes the ecology and physiology of animals. Under the influence of regional variation in climatic factors such as temperature and rainfall animals have evolved strategies that enable them to survive or avoid adverse conditions, and take maximal advantage of favourable conditions. Whilst at the local scale, habitat heterogeneity determines the spatial distribution and ecology of animals through influencing the availability of essential resources.

In seasonal environments, animals are confronted with a number of physiological challenges associated with changes that occur in the environment, and seasonal animals have evolved both physiological adaptations and life history strategies to tolerate this environmental variation. There are three main strategies adopted by animals when faced with seasonally adverse conditions: moving away from adverse conditions, modifying their physiology, morphology or behaviour to survive an inhospitable environment and to utilise torpor.

For some species, the high mortality risks associated with seasonal environments are such that they must migrate away from an area during adverse seasons. Migration can vary from large scale movement from one geographical area to another, which is common in animals with high vagility, such as birds (e.g. Arctic terns (*Sterna paradisaea*) (Egevang *et al.* 2010), swift parrots (*Lathamus discolor*) (Saunders and Heinsohn 2008)), and large ungulates (e.g. elk (*Cervus canadensis*) (Boyce 1991) and caribou (*Rangifer tarandus*) (Ferguson and Messier 2000)), but is rare in small mammals. Seasonal migration may also occur at a much smaller scale. For example a number of Tasmanian birds show altitudinal migration (Ratkowsky and Ratkowsky 1978; Thomas 1987) and white tailed deer (*Odocoileus virginianus*) move from areas of deep snow (Hurst and Porter 2008).

The second strategy used by animals to survive seasonally adverse conditions is to remain in the same area, but to reduce energetic costs by modifying their physiology, morphology or behaviour to enable survival in an inhospitable environment. For example, small mammals, in the months leading up to winter, increase both the density and length of their hair, minimising thermal conductance (Dew *et al.* 1998), which may

be reduced further by growing thick layers of insulating fat (Prestrud and Nilssen 1992). Other species undergo a reduction in body size of as much as 50% and consequently reduce their energy requirements - the Dehnel effect (Lovegrove, 2005). Movements and activity may exploit the presence of snow, with movement and foraging occurring in the subnivean environment, the snow buffering extremes in air temperature (Green and Crowley 1989). Some species may also accumulate stores of energy in the form of food caches during productive periods to supplement reduced resource availability during unfavourable seasons (Humphries *et al.* 2001).

The third strategy employed to survive periods of low energy availability is to utilise torpor. There are two patterns of torpor used by seasonal animals to reduce energetic costs: daily torpor and hibernation (Geiser 1994). During torpor there is a controlled reduction in body temperature and energy expenditure (Geiser 2001). Daily torpor occurs in small animals in response to low food availability and energy restrictions, normally at a definite time of day and season (Geiser and Ruf 1995; Ibuka and Fukumura 1997; Lovegrove and Raman 1998; Perrin and Richardson 2004). Hibernation is long term torpidity, and hibernating species usually reduce their body temperatures to below 10°C (Geiser 2001), with the minimum temperatures of the majority of species being around 5°C (Geiser and Ruf 1995). Hibernation is punctuated by short (usually less than one day) periods of higher body temperatures and intense metabolic activity (Carey *et al.* 2003; Geiser 2001). Torpor and hibernation confer substantial energetic savings to the torpid animal: during torpor metabolic rate is reduced to 2-4% of euthermic rates at similar ambient temperatures (Geiser and Kenagy 1988; Heldmaier and Ruf 1992). During deep torpor the activity of highly metabolically active tissues is reduced and many physiological functions are virtually halted, although essential functions such as respiration and cardiac output continue, albeit at greatly reduced rates (Geiser 2001). A significant contribution to energetic savings during hibernation results from the down regulation and atrophy of the gut (Carey *et al.* 2001; Hume *et al.* 2002). Recovery of intestinal morphology and function is triggered by ingesting food (Hume *et al.* 2002) and may take from two (Vinogradova and Shestopalova 1996) to four weeks (Hume *et al.* 2002). Consequently foraging generally does not occur during periodic arousals and little or no feeding takes place during the hibernation season (Hume *et al.* 2002).

Thus, whilst hibernation confers energetic savings for animals, it requires large energy reserves in the form of fat prior to entering hibernation to fuel extended periods without feeding. Changes in body mass can be dramatic; during prehibernatory fattening, golden mantled ground squirrels (*Callospermophilus lateralis*) and arctic ground squirrels (*Spermophilus parryii*) double their body weight and triple their fat mass (Galster and Morrison 1976), little brown bats (*Myotis lucifugus)* increase their body mass by 38% and almost triple their adiposity (Kronfeld-Schor *et al.* 2000), while dwarf fat tailed lemurs (*Cheirogaleus medius)* (Fietz and Ganzhorn 1999) and woodchucks *(Marmota monax)* (Concannon *et al.* 2001) may double their body mass. Hibernating animals are dependent on these lipid stores to sustain them through the prolonged period of hibernation, and they can also fuel reproduction which usually takes place after emergence. The high energetic demands of reproductive activities may result in further declines in body mass. For males, mass is often lowest after the mating period (Buck and Barnes 1999; Gür and Gür 2005) and for females the energetic burden of lactation may slow rates of fat replenishment (Schwanz 2006). Consequently, these highly seasonal animals are characterised by a large annual fluctuations of body mass (Ebling and Barrett 2008) which reflect food intake, energy storage and metabolism at specific times of the year.

The body mass of eutherian mammals is regulated by a lipostatic system (Diaz *et al.* 1992; Kennedy 1953; Tarasuk and Beaton 1991) involving a number of hormonal signals that have a circulating concentration proportional to body fat reserves and act on the hypothalamus to adjust energy expenditure and food intake (Benoit *et al.* 2004; Rushing *et al.* 2001; Woods and Seeley 2001; Wren *et al.* 2001). One of these hormones, leptin, is a key hormone involved in the long term regulation of body mass, and conveys information on overall nutritional status to the brain (Ahima *et al.* 2000). Leptin is synthesised and secreted primarily by adipose tissue and its primary action is to decrease the hypothalamic production of neuropeptide Y, a neurotransmitter which stimulates appetite and decreases thermogenesis (Ahima and Flier 2000). Elevated deposits of adipose tissue result in an elevated secretion of leptin which, acting on receptors in the hypothalamus, inhibit appetite and increases metabolic rate, leading to a loss of adipose tissue. This anorexigenic signal is removed when body adiposity and associated circulating leptin levels are low, which stimulates food intake and decreases metabolism,

thereby restoring normal energy balance (Ahima and Flier 2000; Houseknecht *et al.* 1998; Schwartz *et al.* 1996). Much research on leptin has focused on the causes and possible treatment of obesity in humans. However, for seasonal animals, the key to survival is not avoiding obesity, but accumulating sufficient fat stores to overcome adverse environmental conditions or food shortages. In these animals the influence of leptin needs to be circumvented to enable seasonally appropriate fattening.

Leptin, or a leptin-like peptide, has been identified in a wide range of taxa. The interaction between leptin and body mass has been studied in detail in eutherian mammals including humans, rodents (Gutman *et al.* 2008; Johnson *et al.* 2004), (Klingenspor *et al.* 2000; Li *et al.* 2004; Zhao and Wang 2006) and Sika deer (Suzuki *et al.* 2004). The presence of leptin, or a leptin-like peptide, has been confirmed in amphibians (Boswell *et al.* 2006; Crespi and Denver 2006), bony and cartilaginous fish (Frøiland *et al.* 2010; Gambardella *et al.* 2010; Kurokawa *et al.* 2005), reptiles (Niewiarowski *et al.* 2000; Paolucci *et al.* 2001; Putti *et al.* 2009; Spanovich *et al.* 2006) and birds (Hen *et al.* 2008; Kordonowy *et al.* 2010). However the activity of leptin and its relationship to body mass is unknown or poorly understood for many of these taxa.

Reproduction in hibernating mammals

During hibernation, metabolic processes including spermatogenesis, foetal development and lactation are slowed or reduced (Barnes *et al.* 1986; Geiser 1996; Racey and Swift 1981). Consequently, reproduction and torpor are thought to be mutually exclusive in mammals, and there is little or no temporal overlap in reproduction and hibernation in most mammals (Kenagy and Barnes 1988). In most hibernating mammals, males generally terminate hibernation before females to allow for regrowth of testes and spermatogenesis, as well as to locate females (Barnes 1996; Kortner and Geiser 1998; Michener 1992). These males are able to forage and regain some body condition prior to mating but in general, species that hibernate, due to the short duration of their active season have a shorter period to engage in ingestive and reproductive behaviours than non-hibernators (Bieber and Ruf 2004; Waterman 1996). Within their short active season, hibernating species must intensively forage to regain essential energy stores for the following period of hibernation, in addition to reproducing and feeding their young. Reproduction and lactation are the most energetically costly events in the annual cycle

(Gittleman and Thompson 1988; Hammill *et al.* 2010; Speakman 2008). There is strong selective pressure on seasonal species for the period of rearing young to coincide with the period of maximum food abundance; Columbian ground squirrels (*Urocitellus columbianus*) synchronise their reproductive period with upcoming peaks in vegetation abundance (Lane *et al.* 2011), parturition in African microchiropteran bat species is timed so that young are weaned just before the period of maximum insect abundance (Cumming and Bernard 1997) and female brown bears with newborn cubs leave the den during the period of increasing productivity (Wiegand *et al.* 2008). If food supply fails or is unreliable, lactation is a strategy that buffers the young from variations in the food supply (Dall and Boyd 2004), but places an energetic burden on females. Consequently it is the period of highest energy demand for mammalian females (Hayssen 1993; Speakman 2008), whilst for males the period prior to mating or during mating incurs the highest energetic costs (Lane *et al.* 2010).

Seasonal animals, home ranges and spatial distribution

An animal's home range needs to provide sufficient resources to fulfil the animal's energetic requirements. The size of animal's home range is strongly influenced by the resources available within it (McLoughlin and Ferguson 2000). In highly seasonal environments, habitat quality at times during the year may be lower than in environments that are less variable. In spite of potentially high pulses in productivity, there will also be periods of very low productivity and thus low food availability, and maximum annual home ranges size will reflect resource availability at periods of lowest productivity (Harestad and Bunnel 1979; Lindstedt *et al.* 1986; McNab 1963; Singer *et al.* 1981). Consequently, more seasonal habitats result in larger home ranges, as animals need to increase the size of their home range to provide enough resources if they are only seasonally abundant (Ferguson *et al.* 2009; Herfindal *et al.* 2005; McLoughlin *et al.* 2000; Nilsen *et al.* 2005). Whilst entry into hibernation may eliminate the necessity of expansion of home ranges during periods of low productivity, like other species, the home ranges of hibernators will still need to provide sufficient energy throughout the active period, but must also encompass sufficient resources to restore highly depleted energy stores following hibernation.

As lactation is a very energetically expensive part of the annual cycle for females (Hayssen 1993; Speakman 2008), it is essential that home range also provides sufficient

resources during this period. Reducing energy expenditure during the period of lactation and prehibernatory fattening is especially important, and home ranges should allow maximal access to resources with minimal effort to increase/enhance success of reproduction and hibernation (Armitage 1998). Accordingly, for many species, the size of female home range is a reflection of habitat quality, with smaller home ranges occurring in more productive habitats (e.g Ferguson *et al.* 2009; Gehrt and Fritzell 1998; Herfindal *et al.* 2005; Mysterud 1999).

Habitat productivity and seasonality is a primary determinant of population density (Saunders 1996; Zalewski and Jędrzejewski 2006) and amount of home range overlap (McLoughlin *et al.* 2000). Excluding conspecifics from home ranges, or portions of home ranges, through active defence is termed territoriality (Powell 2000), and animals are territorial only when a resource is limiting (Brown 1969). In highly productive habitats, exclusive access to resources may not be necessary to secure sufficient resources and conversely, when productivity is low, the costs of defending exclusive access to resources within a territory may not be returned (Carpenter and Macmillen 1976). Consequently, there is likely to be high amounts of overlap in home ranges in both high and low quality habitat, with little exclusive area, and areas of intermediate productivity should result in low overlap of home ranges (McLoughlin *et al.* 2000). Thus interactions between productivity, home range size and population density may determine the spatial organisation of a population.

Territories may be maintained through physical altercations between individuals (Krebs 1982; Stamps and Krishnan 1994) but are generally defended through safer, more energetically economical calls (Cowlishaw 1992; Pollock 1986) , displays (Jullien and Thiollay 1998; Pombal *et al.* 1994) or scent marking (Begg *et al.* 2003; Brashares and Arcese 1999; Gosling 1987). Latrines are frequently used by solitary species to mark territories e.g. (Claridge *et al.* 2004; Rostain *et al.* 2004; Stewart *et al.* 2002). They may also be used by non-territorial species as a site of information exchange, communicating reproductive status (Darden *et al.* 2008), dominance (Sneddon 1991), or enabling partition resource use by signalling where an individual is feeding or quality of food (Begg *et al.* 2003; Henry 1977; Kruuk 1995). Clan based species may also use latrines (Owens and Owens 1978; Henschel and Skinner, 1987).

Study animal and study site

This study examines the influence of seasonal and habitat variation on the energetics, diet, and spatial ecology of the short beaked echidna (*Tachyglossus aculeatus*). The short beaked echidna is the most widely distributed of the extant monotremes and is found across the whole of Australia and in some parts of New Guinea (Griffiths 1968; Strahan and Van Dyck 2008). Echidnas are a highly successful species; found in all habitats from coastal areas to alpine elevations, and do not appear to have any specific habitat requirements other than food and shelter. Echidnas are highly seasonal throughout their range and display annual cycles in energy flux which are coordinated with well defined periods of activity, reproduction and change in body mass (Nicol and Andersen 2007a). They show reduced activity or enter torpor in most areas of their range within Australia. In the Australian Alps and Tasmania, the hibernation season may last for up to eight months and features extended bouts of deep torpor, with body temperatures below 10^{o}C for up to 18 days, between brief normothermic periods that last up to two days (Beard *et al.* 1992; Grigg *et al.* 1992; Nicol and Andersen 1996; Nicol and Andersen 2002). However, unlike most other hibernating species, hibernation in the echidna does not appear to be a response to, or anticipation of cold or absence of food (Nicol and Andersen 1996). Echidnas enter hibernation from late summer to early autumn, when ambient temperatures are still mild, and reproductive males terminate hibernation in early winter before the coldest part of the year (Nicol and Andersen 2007a). Although less abundant during winter, food would be still available with increased foraging effort (Abensperg-Traun and De Boer 1992; Beard and Grigg 2000; Brice *et al.* 2002; Nicol and Andersen 1996; Smith *et al.* 1989). Therefore, the timing and duration of hibernation does not follow a typical mammalian pattern.

The life history of Tasmanian echidnas has recently been summarized by Nicol and Morrow (2012). In brief, echidnas are not reproductively active every year. In non-reproducing years males and females hibernate for approximately six months, entering hibernation during late summer months of February/early March and emerging in October. Before entry into hibernation, the testes of reproductive males increase in size and are at near maximum size when they arouse from hibernation several weeks before the winter solstice (Nicol and Morrow 2012). During the mating season, reproductive males can be readily determined by the presence of a large penis bulge posterior to the

cloaca and the penis may be easily everted (G. Morrow, pers. comm.). After extensively feeding for a period of approximately 25 days, males seek out females, most of which are still hibernating. After mating, opportunities for further feeding for males are reduced as they may guard the female for several days before searching for other opportunities to mate. Mating finishes by October and males focus on foraging. Foraging and weight gain is maximal post reproduction (Nicol and Andersen, 2007a).

The majority of Tasmanian females are still hibernating when found by males, and may re-enter hibernation after being mated. After a gestation period of approximately 18 days the female enters a nursery burrow, and a single egg, 13-17 mm in diameter and weighing approximately 2g (Griffiths 1968), is laid into the pouch several days later (Morrow *et al*. 2009). The egg hatches after about 10 days of incubation (Griffiths 1968). There is limited opportunity for mothers to forage prior to entry into the nursery burrow, and she remains in the nursery burrow for up to six weeks (Morrow *et al.* 2009). After emergence from the nursery burrow, the mother leaves the baby in the burrow to forage, periodically returning to suckle her young. The reproductive status of female echidnas is generally easy to ascertain. Pregnant and lactating echidnas develop an obvious pouch with thick tumescent lips, and regular entrance into the nursery burrow with its characteristic back filled entrance is a reliable indicator of reproductive activity. The young remains in the burrow until weaning during late December – early January when the baby is approximately 150 days old (Nicol and Andersen 2007a). Young are weaned by the mother not returning to the nursery burrow, and there is no indication of any interaction between mother and her weaned young. The dispersal patterns of juvenile echidnas are unknown.

The longevity of free-ranging echidnas is not known, but an individual (of unknown age at capture) lived for 49 years at the Philadelphia Zoo in the USA (Crandall, 1964) and an anecdotal report of free ranging individual living for 45 years has also been recorded (Rismiller 1999) indicating that echidnas are very long lived animals.

The echidna is an opportunistic forager; on mainland Australia the diet primarily consists of ants and termites, with a trend for higher intake of termites in hot, arid areas (Griffiths 1978). In cooler, wetter regions, a higher proportion of ants is consumed (Griffiths 1978), along with scarab beetle larvae, the numbers of which vary seasonally (Harrison 1997; Smith *et al.* 1989). Termites are effectively absent from Tasmania; they

do not construct the large mounds found on mainland Australia and only form small colonies within damp wood (Watson and Gay 1991). The impact of the absence of termites on the diet of Tasmanian echidnas has not been determined. Whilst most populations of echidnas show a reduction in activity during winter, Tasmanian echidnas have a long period of hibernation; reproductively active animals hibernating for, on average, 130 days (Nicol and Andersen 2007a). As a result they have a relatively short period of time in which to reproduce and regain the energy stores necessary for the following hibernation period. In the post reproductive period, body mass of the echidna may increase by up to 35% (Nicol and Andersen 2007a). Whether specific dietary items are required or sought out during prehibernatory fattening is not clear, although (Falkenstein *et al.* 2001) found that the fatty acid composition of the depot fat of echidnas on the New England Tablelands of NSW during the pre-hibernation season was almost identical to that of the most abundant prey species, the ant *Iridomyrmex sp*.

Myrmecophages (ant and termite eaters) like the echidna, share a number of characteristics including a solitary lifestyle, minimal intraspecific aggression, low reproductive rate and extended period of parental care, and a significantly reduced body temperature and metabolic rate (Laurie and Seidensticker 1977). Home ranges of echidnas, like those of other ant-eating species, are also smaller than those of similar sized carnivores and omnivores (Nicol *et al.* 2011). As the body size of ant-eating species increases, their basal metabolic rate falls further below the predicted level (McNab 2000) and their lower overall energetic needs are reflected in reduced home range size relative to body mass (Nicol *et al.* 2011).

Echidnas display a high degree of home-range fidelity (Abensperg-Traun 1991; Augee *et al.* 1992; Griffiths 1989; Nicol *et al.* 2011; Rismiller and McKelvey 1994) and home ranges overlap extensively (Abensperg-Traun 1991; Augee *et al.* 1992; Nicol *et al.* 2011). Despite the diversity of habitats and distribution of prey species, home range size throughout Australia stays relatively constant (Nicol and Andersen 2007a), which may indicate that home range size is an inherent species property and that population density may reflect ecosystem productivity (Makarieva *et al.* 2005). There are significant differences in the home ranges sizes of male and female echidnas, with male home ranges more than twice as large as female home ranges, and with no correlation between body

mass and home range size for either sex (Nicol *et al.* 2011). This difference in home range size suggests that, like many other solitary species, home ranges of female echidnas are determined by resources, whereas male home ranges are based on maximising access to females (Clutton-Brock 1989; Emlen and Oring 1977; Sandell 1989).

Except during the breeding season, echidnas are solitary (Augee *et al.* 1975; Griffiths 1978) and there has been no evidence of any social structure in free ranging echidnas. For many species, localised accumulations of faeces (latrines) provide chemical cues which play a role in communication between individuals and regulate their spatial organisation (Begg *et al.* 2003; Jordan *et al.* 2007; Oakwood 2002; Stewart *et al.* 2002; Wronski and Plath 2010). Echidna latrines have been found in a number of sites within Tasmania (Grove *et al.* 2006; Sprent *et al.* 2006) which may function to control the distribution of echidnas across the landscape.

Study site

The study was carried out on population of echidnas within an area of woodland interspersed with improved and native pastures in Tasmania, Australia (See Figures 1 and 2). This population has been studied since 1996, providing valuable background information for this study on echidna distribution, numbers and seasonal behaviour, particularly the timing of hibernation and reproduction. The site was originally selected because of reportedly high echidna numbers, reasonable proximity to the University, and its location on private property, which means it is not subject to disturbance other than low intensity stock grazing. Because this is the first study of its type in Tasmania we cannot comment on whether this population is representative of echidna populations throughout the state, and the cryptic nature of echidnas means that it is extremely difficult to estimate echidna numbers or densities - the time between consecutive sightings of tagged individuals has been as much as 10 years. One of the aims of the long term work at this site is to provide baseline information for comparison with other parts of Tasmania and mainland Australia.

Limitations of study

All data was collected from free ranging animals that were fitted with radio transmitters, or sampled opportunistically when observed when driving around the site. Although transmitters generally remained attached for a minimum of 12 months there

were still incidences of transmitter loss, battery failure, or animals were not accessible. The result of this was that series of location data and blood samples were often incomplete, and were characterized by periods of relatively consistent data punctuated by often large gaps. Rather than only using data from a single year which may have gaps in the data available, to reduce the impact of these gaps, blocks of data were selected from multiple years that reflected activity throughout the year.

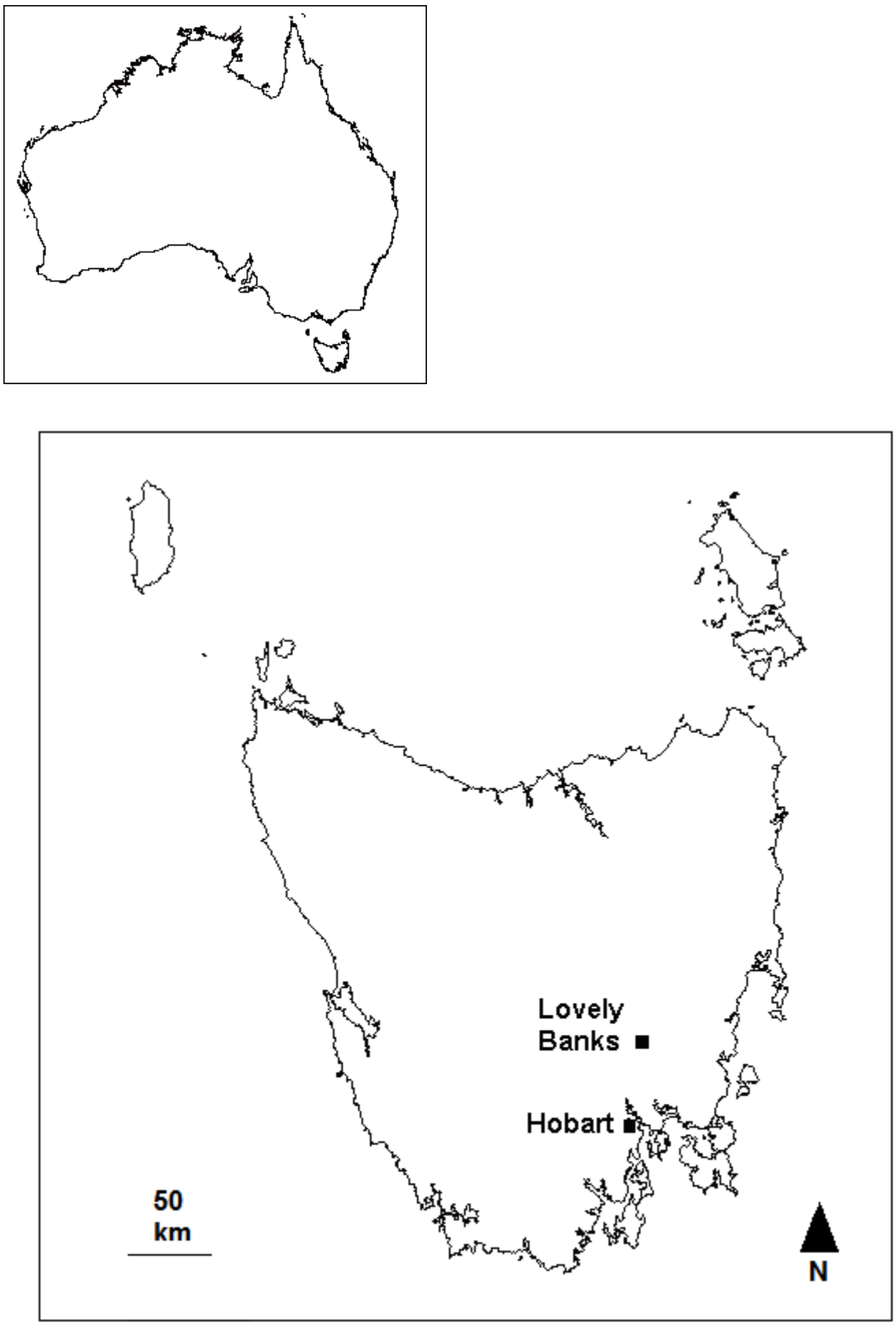


Figure 1. Location of the study site. Fieldwork was carried out at Lovely Banks, a grazing property in the Southern Midlands, 55 km north of Hobart, Tasmania, Australia (longitude 147 14', latitude 42 25'S).

Figure 2. Photo of vegetation in the study area Lovely Banks in the Southern Midlands of Tasmania. Vegetation at the site consists of remnant dry sclerophyll woodland dominated by *Eucalyptus amygdalina* interspersed with improved and native pasture and patches of *Acacia dealbata*, *Casuarina verticilata* and *Lomandra longifolia*. The site is intersected with numerous gullies, caves and sandstone outcrops and ranges in altitude from 200 to 400 m asl.

Thesis Objective

In this thesis I investigate aspects of the seasonal physiology and ecology of Tasmanian echidnas. Tasmania is the most southerly part of the range of these ubiquitous Australian mammals, and the area where seasonal effects are likely to be the greatest, as demonstrated by their long period of hibernation, and large seasonal variation in body mass. This raises a number of questions, such as 1) What is the role of the hormone leptin, the major lipostatic hormone in eutherian mammals, in the regular annual variations of energy expenditure and body mass? 2) Are specific dietary items required or sought out at different parts of the annual cycle? 3) How does the diet of Tasmanian echidnas compare with echidnas in other parts of Australia? 4) Does diet vary between habitat types, and what is the relationship between home range size and habitat type? 5) How do echidnas arrange themselves in the landscape? I attempt to answer these questions in this study.

The following chapters include research on a number of aspects of the physiology and spatial ecology of echidnas, focusing on the challenges and requirements of these seasonal animals. The challenge of gaining sufficient energetic stores in the months prior to hibernation is explored, both in terms of the physiological modifications that enable this mass gain to occur and actual diet consumed throughout the year. Furthermore, I test whether the strong seasonality of the life history of echidnas is reflected in their diet. In order to gain access to sufficient resources for successful hibernation and reproduction, echidnas need to maximise their opportunities for productive foraging. The spatial distribution of home ranges is mapped and the relationship between vegetation type and home range size determined and possible influence of resource availability explored. Lastly, latrines are used by both male and female echidnas, and their possible role in communication between individuals and maintenance of the spatial organisation of echidnas is also investigated.

Thesis Structure

This thesis has been structured as four inter-related papers written in a format to facilitate publication in the near future, and one chapter subsection (Chapter 5a) has been published. Each chapter has been written as a self-contained paper, which has resulted in some repetition and stylistic variation throughout the thesis.

Chapter 2

Echidnas display a clear annual cycle of body mass. Maximum mass occurs just prior to entry into hibernation and mass drops to minimal values at the end of mating. The adipose tissue derived hormone leptin plays an important role in regulating body mass in many other species. I hypothesised that in echidnas, as in eutherian mammals, circulating leptin concentrations will show a positive relationship with adiposity, and this relationship would change during the period of pre-hibernatory fattening, when increased adiposity is adaptive. Adequate energy stores are essential for successful reproduction, especially for female echidnas which, after hibernating for up to six months, mate during winter and then enter a nursery burrow for a further six weeks. I hypothesised that females that are fatter at entry to hibernation would breed in the following reproductive season, and that leptin provides the signal of body condition to the reproductive axis.

Chapter 3

In this chapter I investigate if the strong seasonality of echidna life history is reflected in their diet. Is there any relationship between the presence of specific dietary items and the pattern of hibernation and reproduction? Other detailed studies of echidna diet have been carried out on mainland populations where termites are generally abundant; a prey item which is effectively absent from Tasmania. How does the absence of termites affect the diet of Tasmanian echidnas? Is there any evidence of other non-ant prey in the diet of Tasmanian echidnas? Scat analysis is used to determine actual prey items consumed, and patterns of consumption compared between animals living within difference vegetation types using stable isotope analysis of echidna blood and prey items.

Chapter 4

In chapter 3, the clear differences in the isotopic signatures of $\delta^{13}C$ and $\delta^{15}N$ in the blood of echidnas inhabiting different vegetation types indicate a high degree of home

range fidelity. In this chapter the influence of vegetation type on home range size was investigated. The study site consists of a mosaic of woodland and pasture, and as woodland contains more opportunities for shelter and is likely to have more food resources, I propose that it represents higher quality habitat. I hypothesised that animals in areas with less woodland cover would have larger home ranges in order to obtain sufficient resources. If, like many other solitary species, female home ranges are based on access to resources, but those of males are not, then the relationship between home range size and woodland cover should differ between the sexes. The habitat that forms the interface between two vegetation types (edge habitat) is important in determining home range size in some species, and an alternative hypothesis is that amount of available edge habitat is important in determining the size of the home range.

In order to obtain a better idea of how animals use their habitat GPS loggers were attached to echidnas. GPS loggers have not previously been deployed on echidnas, so this was, in part, a trial to test their future use in investigating the spatial ecology of echidnas.

Chapter 5a/b

In chapter 4, I showed that the type of vegetation in which home ranges are located has a significant effect on the size of home ranges of female echidnas. I hypothesised that higher quality habitats will generally support a higher density of individuals, and as population density varies, so will the amount of interaction between inhabitants. One means of communication in a solitary species such as the echidna may be through the use of latrines, which have been observed at the current field site. These latrines are described in Chapter 5a. Latrines may function in the maintenance of spatial distribution of individuals, or can be used to transfer information regarding social or reproductive status between individuals. Chapter 5b focuses on factors affecting their location and some of the possible roles of latrines in the spatial ecology of echidnas. The distribution of latrines in relation to vegetation type, as well as the spatial distribution of echidnas, was determined, and the possible functions of latrines in social interactions and marking of territorial boundaries discussed.

Chapter 6

The thesis concludes with a general discussion that synthesises the impact of a seasonal environment and habitat variation on the physiology and spatial ecology of echidnas, and summarise possibilities for future research.

Chapter 2

Seasonal variation in circulating leptin concentrations and body mass in free ranging short beaked echidnas (*Tachyglossus aculeatus*)

Formatted for submission to *Journal of Comparative Physiology*

Abstract

Leptin is a peptide hormone best known for its role in feedback regulation of adiposity in mammals. An increase in adipose tissue mass leads to an increase in circulating leptin which increases energy expenditure and limits food intake. In hibernating mammals this relationship may change to allow prehibernatory fattening. The echidna is a monotreme mammal which accumulates significant fat reserves before entering hibernation, with males mating at the end of hibernation and females mating during hibernation. It was hypothesised that echidnas would show a strong relationship between body mass and plasma leptin for most of the year which would change during the pre-hibernatory period. Plasma leptin and body mass in free-ranging echidnas was measured over the reproductive and hibernation cycles. There were significant seasonal variations in plasma leptin in both sexes, with the highest levels occurring in hibernation and in females during mating. The lowest levels were found in males when they were foraging maximally after the reproductive period. Rather than the strong positive relationship between adiposity and plasma leptin found in other mammals, a weak negative relationship was found, as has been found in reptiles and birds.

Introduction

Animals living in temperate and polar latitudes must adjust their behaviour and physiology in response to annual changes in environmental conditions, which may include extremes of temperature, low food availability, high energy demand or a combination of these factors (Bartness *et al.* 2002). Many mammals have evolved an extreme response to seasonality: hibernation, which is a periodic, temporary abandonment of homeothermy, which substantially reduces energy expenditure usually during periods of food shortage or adverse environmental conditions (Geiser 2001). During hibernation, a controlled reduction in body temperature is accompanied by a suite of physiological modifications that help conserve energy, including reduced respiration and heart rates and down-regulation of the gastrointestinal system (Carey 1995). It has been estimated that for a four kilogram mammal, hibernation saves up to 83% of the costs of maintaining euthermy (Armitage *et al.* 2003).

In spite of the significant reduction of metabolic rate that occurs during hibernation, it is invariably accompanied by body mass loss, which is often further exacerbated by the onset of reproductive activities soon after emergence from hibernation (Buck and Barnes 1999; Gür and Gür 2005; Lagarde *et al.* 2002). Predictable annual cycles of body mass and adiposity are dominant features of almost all seasonal animals including migratory birds (Maggini and Bairlein 2010), reptiles (Wapstra and Swain 2001), and mammals (Buck and Barnes 1999; Dark 2005; Kunz *et al.* 1998). Hibernating mammals in particular show a dramatic increase in adiposity in the period leading up to hibernation as a result of increasing their food intake in preparation for an extended period with little or no foraging (Concannon *et al.* 2001; Fietz and Ganzhorn 1999; Florant *et al.* 2004; Kunz *et al.* 1998).

In eutherian mammals, the peptide hormone leptin has a key role in the regulation of fat reserves. Leptin is synthesised and secreted primarily by adipose tissue, and an increase in adiposity in eutherian mammals is normally associated with a corresponding increase in the synthesis and secretion of leptin by adipocytes, resulting in increased circulating leptin concentrations (Sahu 2004). Leptin binds to leptin-specific receptors in the hypothalamus, regulating the production of a range of orexigenic and anorexigenic neuropeptides, and resulting in a decrease in food intake, an increase in metabolic rate, and consequently a loss of adipose tissue (Ahima and Flier 2000; Houseknecht *et al.* 1998; Schwartz *et al.* 1996).

However, for species which must store large amounts of adipose tissue in preparation for hibernation, these effects of leptin would be inappropriate during pre-hibernatory fattening. For much of the year, many eutherian hibernators show the same close relationship between the amount of adipose tissue and circulating leptin concentration as non-hibernators (Boyer and Barnes 1999; Florant *et al.* 2004), when prevented from hibernating by maintaining ambient temperatures above 20°C, woodchucks (*Marmota monax*) display this close relationship throughout the year (Concannon *et al.* 2001). To prevent feedback inhibition of feeding during seasonal fattening two mechanisms are used by hibernators and strongly seasonal eutherian mammals: down-regulation of hypothalamic leptin receptors (leptin resistance) and down-regulation of leptin gene expression in white adipose tissue (leptin decoupling). In the former case, circulating leptin concentrations correlate with adiposity but receptors

are resistant to the effects of elevated leptin concentrations (Atcha *et al.* 2000; Rousseau *et al.* 2002); in the second the relationship between circulating leptin concentrations and adiposity becomes uncoupled (Townsend *et al.* 2008). For example, in the hibernating Djungarian hamster (*Phodopus sungorus*) (Scherbarth and Steinlechner 2010) annual changes in body weight and fat mass are closely correlated with leptin concentrations in the blood (Korhonen *et al.* 2008). Hamsters respond to exogenous leptin during autumn and winter but in spring and summer are virtually leptin-resistant (Atcha *et al.* 2000; Klingenspor *et al.* 2000; Rousseau *et al.* 2002). High leptin concentrations during spring and summer do not evoke anorexigenic effects because the hypothalamic leptin receptors are down-regulated. A similar mechanism has been observed, or postulated, in hibernating marmots (*Marmota flaviventris*) (Florant *et al.* 2004) and field voles (*Microtus agrestis*) (Król *et al.* 2006).

Some hibernators show a dissociation of plasma leptin concentrations from adiposity. In little brown bats (*Myotis lucifugus*) plasma leptin concentration increases before adiposity, and then decreases at entry to hibernation, although adiposity remains high (Kronfeld-Schor *et al.* 2000; Townsend *et al.* 2008). Similar patterns of leptin concentrations and adiposity have been observed in the raccoon dog (*Nyctereutes procyonoides*), a canid omnivore which shows autumnal fattening and then spends 4-5 months in shallow torpor in a den (Asikainen *et al.* 2004; Nieminen *et al.* 2002), and the blue fox (*Alopex logopus*) which, although winter active, shows strong seasonal variations in physiology, with mass and metabolic rate being minimal during winter (Fuglei *et al.* 2004; Fuglei and Øritsland 1999; Mustonen *et al.* 2005).

The highly seasonal short-beaked echidna, *Tachyglossus aculeatus*, is found across mainland Australia and offshore islands including Tasmania and parts of Papua New Guinea (Augee 2008). Echidnas living in the colder areas of the species' range hibernate, and like other hibernators, show large seasonal variations in all aspects of their energetics. In Tasmania, echidnas reduce activity and hibernate for up to 8 months of the year: body temperature drops to within 0.5^{o}C of soil temperature (Nicol *et al.* 2004), and metabolic rate drops to one twentieth of field metabolic rate (Nicol and Andersen 2007a). There is a strong annual cycle of body mass. Mass is at its maximum just prior to entry to hibernation and at its lowest at the end of mating for males, and for females, after exiting the nursery burrow. Sexually mature adult males show a seasonal variation in

body mass of about ± 25% and a variation of about ± 30% for females (Nicol and Andersen 2007a). Reproduction is strictly seasonal and individual females do not mate every year; in some years they hibernate through the mating season (Nicol and Andersen 2002). Mating occurs from mid July to early September, soon after male arousal from hibernation, and may actually overlap with female hibernation as males may mate with females which are still hibernating (Morrow and Nicol 2009). The rate of mass loss is approximately four times greater during reproduction than hibernation (Nicol and Andersen 2007a).

Hibernation in echidnas appears to be a strategy that reduces energy expenditure during times of relatively low food availability rather than because of absolute energetic necessity. In Tasmania, echidnas enter hibernation in late summer when temperatures are still relatively warm, and food available (Nicol and Andersen 1996), and arouse in mid winter to mate when temperatures are minimal (Morrow and Nicol 2009). In the Australian Alps and New England Tablelands animals may still remain active in sub-zero temperatures (Grigg *et al.* 1992; Smith *et al.* 1989). While food may be less abundant during winter, it does not appear to be limiting: sufficient food would be available with increased foraging effort (Abensperg-Traun and De Boer 1992; Nicol and Andersen 1996; Smith *et al.* 1989). Once they reach their maximum mass in late summer, rather than continuing to forage to maintain good body condition, echidnas allow their body temperature to fall to near ambient levels, reducing metabolic rate and thus conserve their energy stores for use during reproduction (Grigg and Beard 2000).

The monotreme and therian mammal lines diverged 166 million years ago (Warren *et al.* 2008), and many aspects of monotreme reproductive biology align them more closely with reptiles and birds than mammals (Renfree 1995). There are also differences in energetics between monotremes and other mammals: monotremes have a low body temperature, low resting metabolic rates (Dawson *et al.* 1979), and, unlike eutherian hibernators, echidnas lack brown adipose tissue, limiting their rate of heat production during rewarming (Nicol *et al.* 2009). Thus, the role of leptin in the regulation of seasonal variation in energy balance in echidnas is of considerable interest. Leptin appears to be highly conserved in vertebrates; its presence has been verified in a wide range of taxa and, while in all species studied leptin appears to play a role in the

regulation of energy balance (Sahu 2004), a positive relationship between adiposity and plasma leptin is not seen in reptiles and birds.

The aim of this study was to investigate annual variation of serum leptin concentrations of free ranging echidnas, and investigate the relationship to the seasonal variations in body mass, and to the reproductive cycle. It was hypothesised that, in echidnas, as in most eutherian mammals, there would be an annual cycle of circulating leptin with leptin levels being directly proportional to adiposity during most of the year, but that a change in this relationship would occur during the period of prehibernatory fattening, when increased adiposity is adaptive.

In addition to having a direct feedback control of adiposity, in some eutherian mammals leptin may have an important role in female reproductive function. Low circulating leptin concentrations, associated with suboptimal energy stores, may inhibit reproduction (Schneider *et al.* 2000). As echidnas do not breed every year, it was hypothesised that females that were fatter at entry to hibernation would be more likely to breed than lighter females, and that leptin would provide the signal of body condition to the reproductive axis.

Materials and Methods

Animal capture and blood sampling

Fieldwork was carried out at Lovely Banks, a grazing property in the southern midlands, 55 km north of Hobart, Tasmania (longitude 147 14', latitude 42 25'S). During the period 1997 – 2009 up to 12 adult echidnas in any year had a radio transmitter (Biotelemetry Tracking, St Agnes, South Australia) glued to their lower spines using 2-part epoxy glue. Transmitters weighed 20g which is less than 1% of total body mass of an adult echidna. An attempt was made to recapture animals every two months; however this was not always possible due to loss of tracking transmitters and animals taking refuge in inaccessible locations. Activity at the time of capture was recorded as falling into one of three categories: *hibernating, mating* or *active* (but not involved in mating). Once animals were captured, their body mass was recorded. To explore the relationship between female body mass and adiposity at entry to hibernation and behaviour in the following reproductive period, the reproductive status of females was determined. Because reproductively mature female echidnas do not participate in reproductive activity every

year (Nicol and Andersen 2002), the reproductive status of females was assessed from the presence or absence of a well defined pouch, observations of mating, or use of a nursery burrow.

Blood was taken from the rostral sinus under light isoflurane-oxygen anaesthesia. Using 26 1/2 gauge needles, approximately 1 ml of blood was drawn into a syringe (Nicol *et al*, 2005) and placed on ice until the sample was processed in the laboratory on the same day. In the laboratory, blood samples were centrifuged at 6000 rpm for approximately 10 minutes, gently stirred to break up any fibrin clots and centrifuged for a further 10 minutes. The serum was then drawn off with a pipette and stored at -20^0C until analysis.

Radioimmunoassay

Leptin concentration was determined in 100µL blood serum using a commercial multi-species leptin radioimmunoassay (RIA) kit (Cat. # XL-85K, Linco, St Charles, USA) in accordance with the manufacturer's recommended procedure. The antibody used in the kit was raised against human leptin, but displays broad cross reactivity to leptin of many other species including rats, pigs, horses, cows, sheep, bats and ground squirrels (Linco Research Procedure guidelines, 2004).

Western Blot analysis

In order to verify the presence of leptin in echidna adipose tissue, western blot analysis was carried out using adipose tissue taken from a fresh road-killed echidna along with adipose tissue from a laboratory rat as a positive control. Tissue was rapidly frozen in liquid nitrogen, powdered and stored at -80°C. Powdered tissue was homogenized in RIPA buffer: (50 mM Tris pH 7.4, 150 mM NaCl. 1 mM phenyl methane sulfonyl fluoride (PMSF, 1 mM), ethylenediamine tetra acetic acid disodium (1 mM, EDTA), aprotinin (5 µg/ml), leupeptin (5 µg/ml), 1% v/v Triton X-100, 1% v/v Na deoxycholate, 0.1% v/v sodium dodecyl sulfate (SDS) (all reagents from Sigma, USA). Protein concentrations were determined using a commercial detergent-compatible total protein assay kit (DC Protein Reagent Kit, Bio-Rad, CA, USA). Briefly, the prepared protein samples in RIPA buffer were diluted 1 in 5 with water, then 25 µl of the diluted sample was added to 125 µl of Reagent A followed by 1 ml of Reagent B. Samples were mixed, incubated at room temperature for 15 min, and absorbances measured

spectrophotometrically at 750 nm. Unknown absorbances were compared to absorbances derived from a set of albumin standards (bovine albumin Type V, Sigma) assayed in the same protocol. 40 μg total protein was separated on 10-12% polyacrylamide gel (SDS-PAGE) then electroblotted for 2 hr onto 0.2 μm polyvinylidene difluoride (PVDF, Bio-Rad, CA, USA) membranes, which were then blocked overnight in blocking solution (0.5% v/v skin milk powder). Leptin protein was probed with the same anti-guinea pig leptin antibody used in the radioimmunoassay of echidna blood (1:100, Cat. # XL-85K, Linco, St Charles, USA) in blocking solution. Antibody conjugates were subsequently detected with a rabbit anti-guinea pig-HRP antibody (1:5000 Millipore, MA, USA) and ECL© chemiluminescence reagent (Pierce, IL, USA).

Data selection and statistical analysis

There were significant differences in mass between individual adult echidnas, with both adult males and females showing a large seasonal variation in body mass (Nicol and Andersen 2007a). Appendix 1 shows details of mass variation of 43 adult echidnas in the study population, including the individuals used in this study. Mean masses used for calculating mass% were obtained over 8.4 $\pm$ 4.3 years (mean $\pm$ SD), range 2-17 years). Once echidnas reach reproductive maturity there is no further significant increase in body mass. Although there are significant mass differences among individuals, and between years for certain individuals, within year fluctuations account for most of the variance in body mass (see Appendix 1). To allow for comparison of within year variations between individuals, a long term mean mass was calculated for each echidna. Individual body mass values were converted to a percentage of the long term mean. This value (mass%) was used as an index of the relative body condition at the time of sampling.

Statistical analyses were performed using the software package Statistica 6.1 (Statsoft, Tulsa, Oklahoma). Data were tested for normality using the Shapiro-Wilks *W* test. All data were normally distributed so parametric tests were used for all analyses. Both male and female echidnas can differ significantly in physiological condition between years – females appear to be unable to successfully raise young in successive years (Rismiller and McKelvey 2000; G. Morrow pers. comm.) and males may not mate every year (Pers. Obs.). Many animals also show significant between year mass differences (Appendix to Chapter 2) and so data from individual echidnas from different

years were treated as though they were different animals, i.e. as being statistically independent. In order to investigate seasonal variations in leptin and mass%, and any relationships with reproduction, samples were classified as falling into one of three periods: prehibernation (*prehib*, weeks 2-10 of the calendar year, which is the period just prior to hibernation (Nicol and Andersen 2002)), reproduction (*repro*, weeks 23 -36; 98% of matings observed at the field site occurred during this period (Morrow *et al.* 2009)), and post-reproduction (*postrep*, weeks 40 -50, see figure in Appendix to Chapter 2). Samples collected outside these periods were not used in this analysis. Annual variations in plasma leptin and mass% were investigated using factorial ANOVA*,* with sex and period as categorical predictors. When there was more than one data point for an individual animal in a given period in any year, the values were averaged. This resulted in no individual contributing more than one sample per period. To investigate any effect of the activity of the animal at the time of sampling, the three broad categories (*active, hibernating, mating*) recorded at capture (see methods) were used, and the relationship between activity and plasma leptin and mass%, was analysed using factorial ANOVA with sex and activity as categorical predictors. As with period, when there was more than one data point for an individual for a particular activity in a particular year, values were averaged. Significant differences between groups were explored using unequal N HSD tests. The relationship between mass% and plasma leptin for male and female echidnas for different periods was explored using the homogeneity of slopes procedure (ANCOVA) in Statistica, with mass% as a continuous predictor and period as a categorical predictor. The overall relationship between mass% and plasma leptin was explored using simple linear regression with mass% as the single continuous predictor variable. Comparisons of mass% and circulating leptin concentrations between breeding and non-breeding females were made using unpaired *t*-tests. Unless otherwise stated, values are given as means ± SEM.

Results

Western blotting

A single immunoreactive band located at approximately 16kDa was revealed in echidna adipose tissue and in adipose tissue from a laboratory rat (Figure 1). This indicated the presence of a protein in echidna adipose tissue that has the same mobility as

leptin, and that reacts with an antibody that recognises a range of leptins, including human leptin.

Variation in plasma leptin concentrations and body mass% with period and activity

A total of 197 blood samples were collected between 2003 and 2009 from 19 female echidnas (111 samples) and 15 male echidnas (86 samples) from across the entire study site. When data points for periods and activity were averaged to eliminate duplicates there were 89 samples for analysis of the effect of period (55F, 34M) and 97 samples for analysis of the effect of activity on circulating leptin concentrations (56F, 41M). For the majority of animals, most data was collected from a single year or two consecutive years.

The relationship between plasma leptin, mass% and period for male and female echidnas is summarised in Figure 2. The factorial ANOVA for the effects of sex and period on plasma leptin showed a significant difference between sexes ($F_{1,83} = 7.59$; $P < 0.05$) and a highly significant effect of period ($F_{2,83} = 10.59$; $P < 0.01$). Post-hoc analysis showed that plasma leptin concentrations in *repro* females (3.45 ± 0.31 ng.ml^{-1}, $n = 13$) were significantly higher than in other groupings ($P < 0.05$). *Postrepro* males had the lowest plasma leptin (0.93 ± 0.37 ng.ml^{-1}, $n = 9$). For mass% the factorial ANOVA showed no difference between sexes ($F_{1,83} = 0.55$; $P = 0.46$), and a highly significant effect of period. ($F_{2,83} = 1375$; $P < 0.001$). The highest mass% was observed in *prehib* females ($109.5 \pm 2.10\%$, $n = 25$), and the lowest in *repro* females ($92.91 \pm 2.91\%$, $n = 13$).

When animals were classified on the basis of activity at the time of capture, factorial ANOVA for the effects of sex and activity on plasma leptin showed no differences between sexes ($F_{1,91} = 2.84$; $P = 0.1$), but a significant effect of activity on plasma leptin ($F_{2,91} = 13.51$; $P < 0.001$). As there was no significant difference in leptin concentrations between males and females, the data was combined. One-way ANOVA again showed a significant effect of activity on plasma leptin ($F_{2,94} = 10.91$, $P < 0.001$), with hibernating animals (3.60 ± 0.31, $n = 15$) having a significantly greater leptin concentration than active animals (1.99 ± 0.16, $n = 56$).

The relationship between plasma leptin concentration and mass%

Analysis of covariance showed no effect of period on the relationship between mass% and plasma leptin concentration for males ($F_{2,28} = 0.61$, $P = 0.55$). For females the relationship between leptin concentrations and mass% varied between periods ($F_{2,49} = 3.70$, $P < 0.05$). For the combined data set (male and female, all periods) there was a significant weak negative relationship between plasma leptin and mass% (Figure 3) (leptin = 4.66 – 0.025·mass%, $r^2 = 0.062$, $P = 0.012$, $n = 89$).

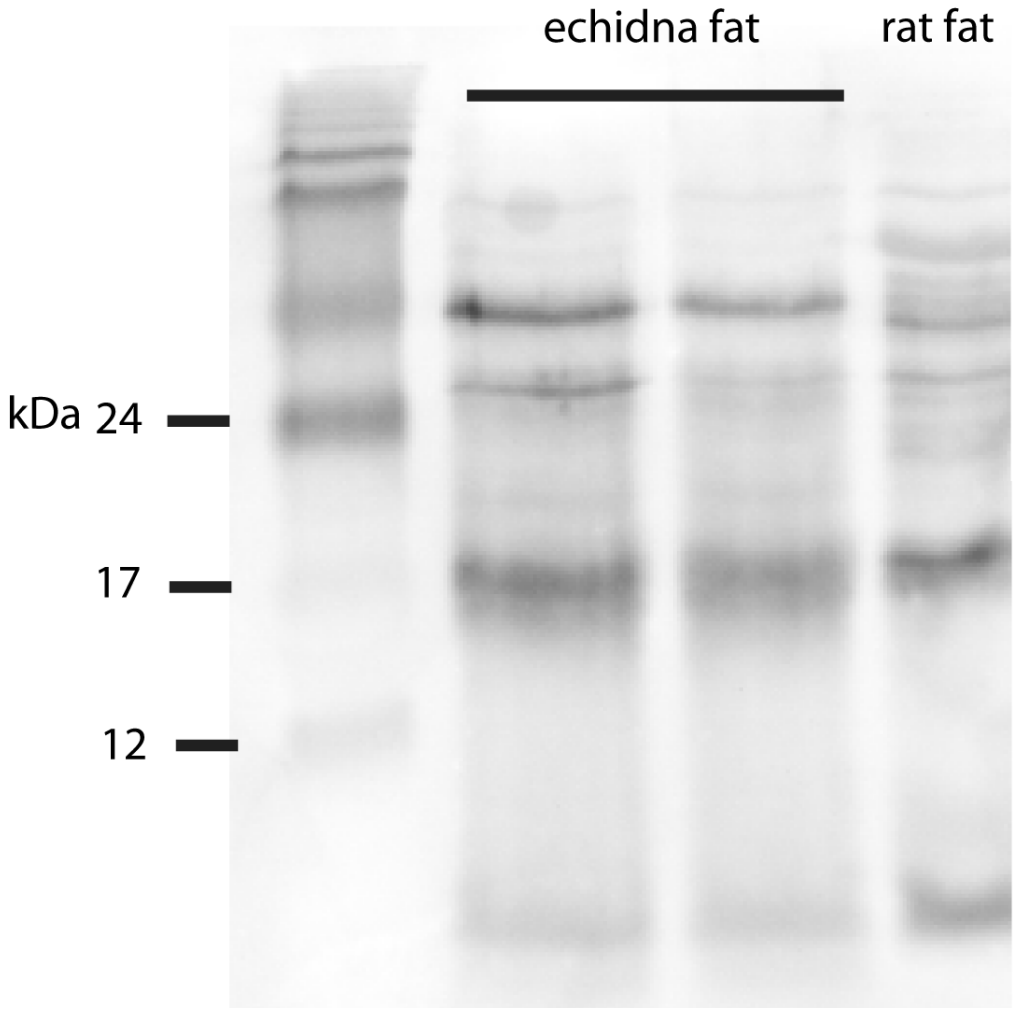


Figure 1 Western blot analysis of echidna adipose tissue, showing a single immunoreactive band at approximately 16kDa, corresponding to the molecular weight of leptin, which co-migrated with recombinant guinea pig leptin. A similar band was also observed in rat adipose tissue. The protein standard (reference band) used to determine the molecular weight of leptin proteins is shown in the left hand lane with molecular weight markers.

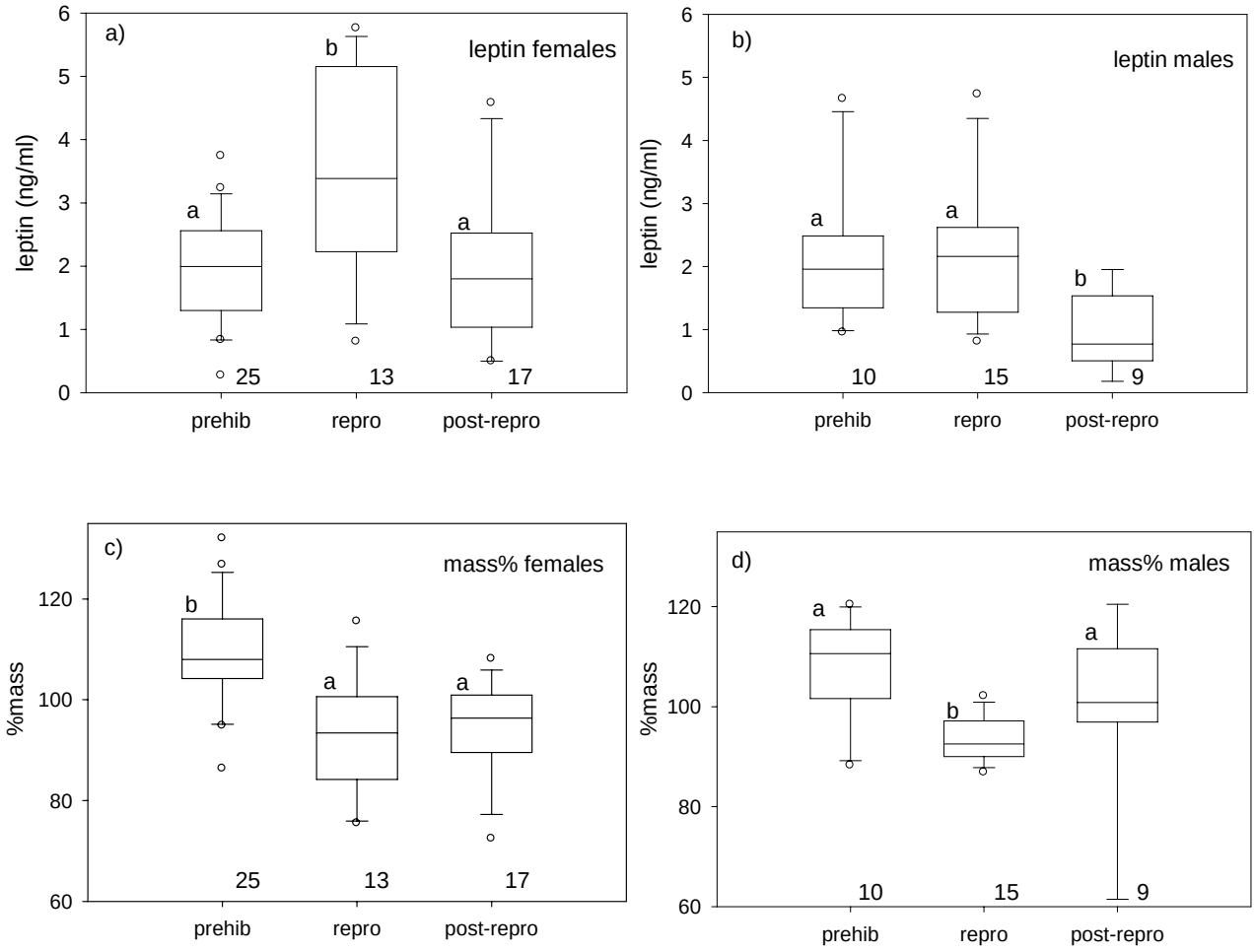


Figure 2: Box plots showing median circulating leptin concentrations during pre-hibernation, reproduction and post reproduction periods for (a) female and (b) male short beaked echidnas (*Tachyglossus aculeatus*). Mass% during pre-hibernation, reproduction and post reproduction periods of (c) female and (d) male echidnas. Numbers refer to the number of animals in each season – only one sample has been included from each animal in each season. Boundaries are 25th and 75th percentiles, and error bars are 90th and 10th percentiles. Circles are outlying points. Plots with the same letter are not statistically different at the 5% level.

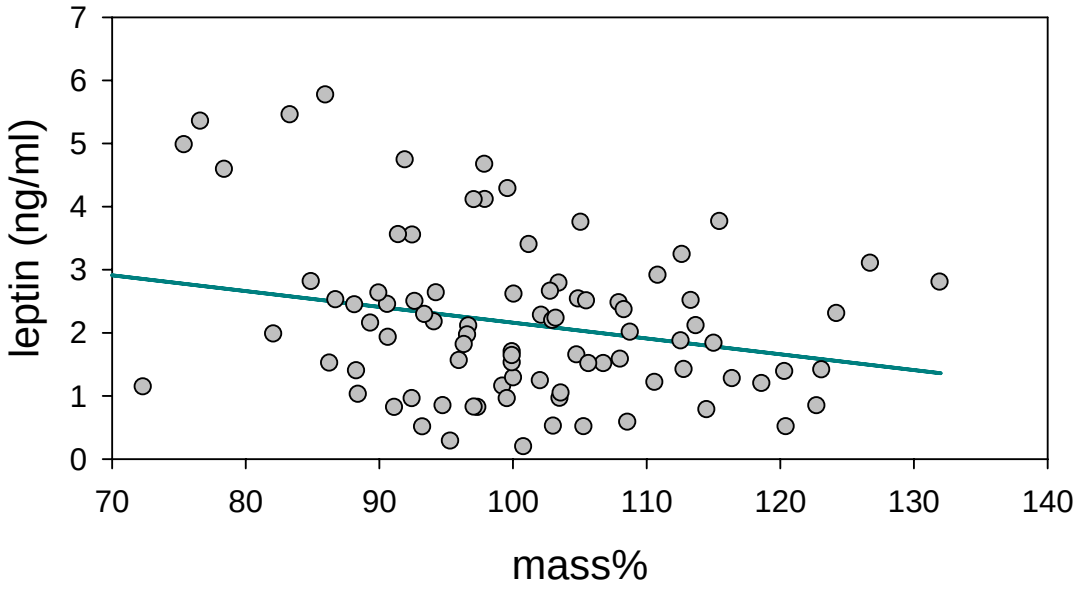


Figure 3: Relationship between mass% and circulating leptin concentration of male and female short-beaked echidnas (*Tachyglossus aculeatus*) for data set shown in Fig. 2. leptin = 4.66 – 0.025·mass%, $r^2 = 0.062$, $P = 0.012$, $n = 89$.

Body mass, plasma leptin concentration and female reproductive activity

Over the period of the field study, 20 occurrences of reproductive activity by adult females were recorded, and 15 occurrences where females hibernated through the breeding season although they were reproductively mature i.e. they had been involved in reproductive activity in previous years. Mean mass of breeding females at entry to hibernation (*prehib* period) was 4.158 ± 0.101 kg, while mean *prehib* mass of non-breeding mature females was 3.67 ± 0.155 kg ($t_{33} = 2.776$, $P < 0.01$). This difference in mass was more significant when expressed as mass%: breeding females 118.9 ± 2.2%, non-breeding 107.3 ± 2.2%, $t_{33} = 3.63$, $P< 0.001$. Blood samples for plasma leptin analysis during *prehib* were available from only 10 of the reproductive females, and 9 of the non-reproducers. The pattern of mass in this subset was similar: mean mass of breeding females was 4.03 ± 0.116 kg; non-breeding 3.58 ± 0.129 kg, $t_{17} = 2.62$, $P < 0.05$. The mean *prehib* plasma leptin concentration in reproductive females was 2.33 ± 0.25 $ng.ml^{-1}$, not significantly different from non-breeding females: 2.22 ± 0.57 $ng.ml^{-1}$, $t_{17} = 0.183$, $P = 0.857$.

Discussion

These results show that while leptin is present in the adipose tissue and plasma of echidnas, circulating leptin concentration does not provide a signal of adipose tissue mass. Plasma leptin shows significant seasonal variations in both male and female echidnas, which correspond with different stages of the reproductive cycle but generally the highest leptin levels occur when body mass is at its minimum (Fig. 2).

As Western blotting indicates that the anti-guinea pig leptin antibody in the Linco Multispecies kit reacts with a protein in echidna adipose tissue that has the same mobility as leptin (Fig. 1), it can be confidently assumed that leptin in echidna plasma was measured. The leptin molecule is strongly conserved (Niv-Spector *et al.* 2005) and the Linco multispecies RIA kit detects leptin in a range of mammal species including bats (Roy and Krishna 2011) and fur seals (Arnould *et al.* 2002), as well as chickens (Dridi *et al.* 2000), and mammalian (anti-mouse) leptin antibodies have been shown to react with lizard leptin (Spanovich *et al.* 2006). In chickens the kit underestimated leptin compared to a chicken leptin-specific RIA (Dridi *et al.* 2000), but while it is quite likely that the estimated leptin values are lower than the true circulating levels, there is no reason to doubt the pattern of variation.

Although adiposity was not measured directly, I am confident that mass% (body mass expressed as a percentage of long-term mean mass) provides a reasonable index of adiposity. When calculating the mean annual mass of individuals, masses were only included after the animals had reached sexual maturity (as evidenced by observation of participation in reproductive activities). After reaching sexual maturity, echidna body mass shows large (25-30%) annual variations about a relatively constant long-term mean (Appendix to Chapter 2; Nicol and Andersen 2007a). Using doubly labelled water to estimate percent body fat in free-ranging echidnas (Green *et al.* 1992) showed close correlation between annual mass variation and body fat. Autopsy of road killed echidnas, and observations during surgical implantation of temperature loggers during an earlier study (Nicol and Andersen 2002), showed these seasonal fluctuations in body mass are associated with changes in the amount of subcutaneous and intra-abdominal fat, which are at their minimum when body mass is lowest, and at their maximum at maximum body mass.

Whilst in most mammals there is a strong, positive relationship between adiposity and circulating leptin, these results show a weak negative relationship between adiposity and plasma leptin concentration in the echidna (Fig. 3). Some other mammals show a departure from a positive relationship during seasonal fattening: in little brown bats there is no corresponding increase in circulating leptin levels during the prehibernatory fattening period despite an increase in body mass (Kronfeld-Schor *et al.* 2000; Townsend *et al.* 2008), and reindeer do not show a rise in circulating leptin levels as mass is regained in spring after winter food restriction (Soppela *et al.* 2008). However, these species show a positive relationship between adiposity and circulating leptin concentrations during the remainder of the year, while in the echidna there is no indication of such a relationship at any time of the year.

In eutherian mammals, in addition to signalling the state of adipose tissue stores, leptin is known to have range of other actions, particularly on reproduction (Govic *et al.* 2008; Schneider 2006; Schneider *et al.* 2007). Leptin administration to food deprived hamsters resulted in an increase in sexual behaviours and a decrease in ingestive behaviours, indicating that leptin may be involved in diverting attention from food towards reproductive behaviours (Ammar *et al.* 2000) and enhancing sexual motivation at appropriate times (Schneider 2006; Schneider *et al.* 2007). The data are consistent with leptin having such a role in the echidna. The highest leptin concentrations are associated with hibernation and, for females, reproduction (Fig. 2). During these periods animals show minimal activity, low body temperatures and do not feed (Green *et al.* 1992; Morrow *et al.* 2009; Morrow and Nicol 2009). In hibernating echidnas, high leptin concentrations may inhibit foraging and thus allow animals to cease activity and enter hibernation. In the early part of the breeding season, when circulating leptin concentrations are high, female Tasmanian echidnas re-enter hibernation between mating events (Morrow *et al.* 2009; Morrow and Nicol 2009). A facilitation of torpor by high circulating leptin is however quite different from what is seen in a range of animals treated with exogenous leptin: although fence lizards showed reduced activity and food intake, they select higher body temperatures (Niewiarowski *et al.* 2000), and in the marsupial *Sminthopsis macroura* (Geiser *et al.* 1998) and eutherian Siberian hamster (Freeman *et al.* 2004) leptin treatment inhibits entry into daily torpor. However, chronic leptin administration to cold-acclimated rats reduces their food intake and thermogenesis

(Abelenda *et al.* 2003), which is consistent with the results of the current study, and acute pharmacological effects may well differ from chronic and physiological responses.

During the reproductive period, male echidnas spend a large proportion of their time finding, mating with, and guarding females, despite their energy stores having been depleted during hibernation. Foraging is infrequent or absent, and during this period males reach their lowest body mass (Nicol and Andersen 2007a). Similar to females, the relatively high circulating leptin levels during reproduction may facilitate reproductive behaviour while inhibiting foraging and eating, leading to greater reproductive success. In the post-reproductive period male leptin is at its lowest and during this period male echidnas forage maximally and show their greatest increase in mass (Nicol and Andersen 2007a).

In the study population most female echidnas become pregnant while still torpid, and even when pregnant are harassed by males until they enter the nursery burrow (Morrow *et al.* 2009; Morrow and Nicol 2009). Because of their limited opportunity to feed and replenish energy stores immediately after arousal from hibernation, I hypothesised that females with higher adiposity prior to entry to hibernation would be more likely to breed in the following reproductive season, which was confirmed by the results. Thus, as in eutherian hibernators (Bieber and Ruf 2004; Cronin and Bradley 1988; King *et al.* 1991), adequate fat stores are critical for reproductive success in the echidna, but there is no evidence that circulating leptin levels are involved in signalling information on energy stores to the reproductive axis in this species.

Leptin has been identified in the blood and other tissues of a wide range of taxa including amphibians (Boswell *et al.* 2006), fish (Gambardella *et al.* 2010), reptiles (Paolucci *et al.* 2001; Spanovich *et al.* 2006), birds (Quillfeldt *et al.* 2009) and eutherian mammals (Zhang *et al.* 1994). Exogenous leptin administration results in the reduction of food intake in amphibians (Crespi and Denver 2006), fish (Murashita *et al.* 2008), reptiles (Niewiarowski *et al.* 2000), birds (Lõhmus *et al.* 2003), marsupials (Hope *et al.* 1999) and eutherian mammals (Halaas *et al.* 1995). In free ranging lizards (Paolucci *et al.* 2001; Spanovich *et al.* 2006) and little brown bats (Kronfeld-Schor *et al.* 2000; Townsend *et al.* 2008), as in the monotreme echidna, low circulating leptin concentrations coincide with periods of intensive foraging. Because of its possible role in the pathogenesis of human obesity leptin is primarily thought of as an afferent signal in a negative-feedback loop

regulating adipose tissue mass (Friedman and Halaas 1998), but it has a range of other actions in the regulation of energy balance, and a strong positive relationship between circulating leptin concentration and body condition is seen only in eutherian mammals and domesticated quail and chickens (Lõhmus *et al.* 2006; Shi *et al.* 2006), but these birds may not be representative of the normal physiological role of leptin in birds in general as they are selectively bred for rapid growth and have very high amounts of body fat (Shi *et al.* 2006). Free ranging birds (Kordonowy *et al.* 2010; Quillfeldt *et al.* 2009), reptiles (Spanovich *et al.* 2006), fish (Frøiland *et al.* 2010) and echidnas all display a weak negative or no relationship between leptin concentrations and body mass, while variations in leptin concentrations are associated with seasonal variations or reproductive cycle. By contrast to its role in adiposity signalling, the anorectic effect of leptin on food intake appears to be the same for all taxa in which it has been examined, suggesting that, in evolutionary terms, this function preceded the role of adiposity signal.

Although leptin is produced in a ranges of tissues, including liver, brain, stomach, testes and pancreas, in reptiles, birds and eutherian mammals the primary site of leptin production is adipose tissue (Niewiarowski *et al.* 2000; Paolucci *et al.* 2001; Putti *et al.* 2009; Quillfeldt *et al.* 2009; Zhang *et al.* 1994). Although the expression of leptin in other tissues in the echidna was not investigated, it seems unlikely that monotremes would depart from this pattern. Although exogenous leptin suppresses feeding in the marsupial *Sminthopsis crassicaudata* (Hope *et al.* 1999), there is no published data on the relationship between adiposity and circulating leptin in marsupials. Thus the adipostatic function of leptin may be common to therian mammals, which separated from the monotreme line approximately 166 mya, or be restricted to eutherian mammals, which diverged from the marsupial line 148 mya ago (Warren et al., 2008). It is tempting to speculate that the evolution of this role of leptin may be associated with the development of brown adipose tissue, which although identified in marsupials (Jastroch *et al.* 2008), reaches its maximum expression in eutherian mammals (Hayward and Lisson 1992).

Appendix to Chapter 2

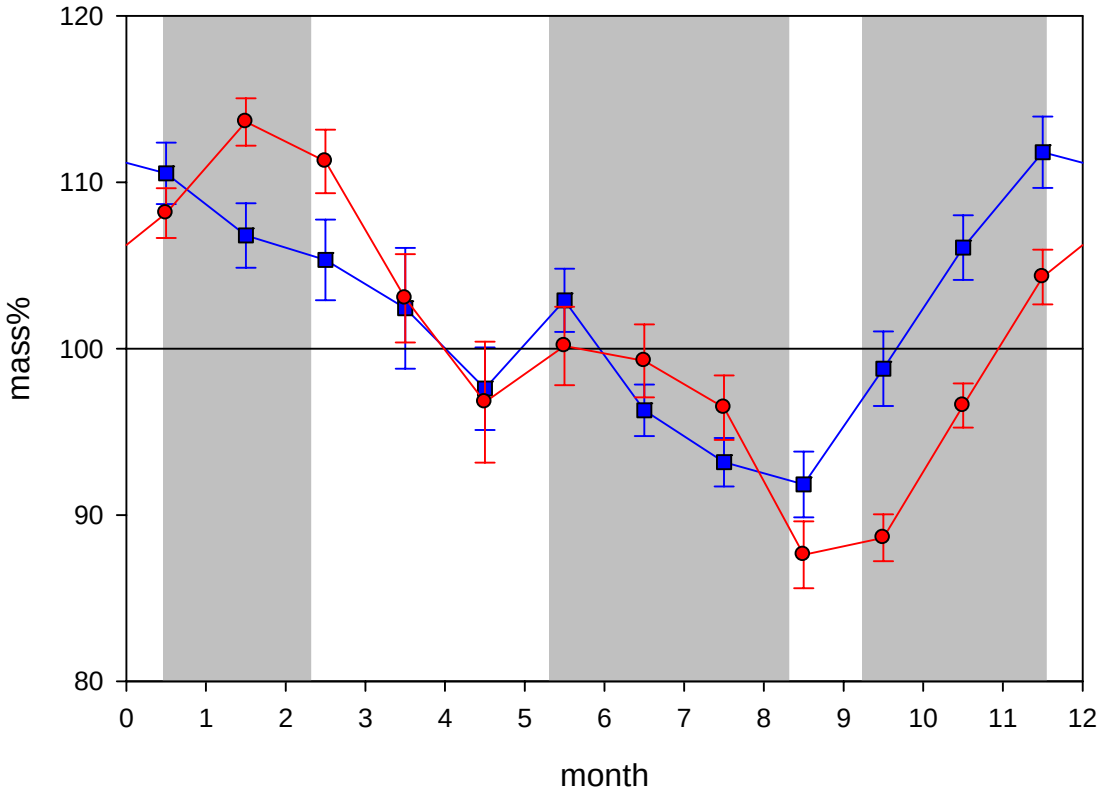
Annual variation in body mass of short-beaked echidna (Tachyglossus aculeatus)

Appendix – Table 1. Summary of mass variation in 43 echidnas (*Tachyglossus aculeatus*) from the study population. Total time: time from first to most recent mass reading. Regression adj r^2, F, slope and P value: result of linear regression of mass against date; year F, P: result of ANOVA of mass and year; period F, P: result of ANOVA of mass and period. n = number of data points used for the regression and ANOVA of mass and year, n*= number of data points used for ANOVA of mass and period.

Data from 5 echidnas show a significant relationship between body mass and date, but in 3 of these the slope is negative. Analysis of variance of the whole data set for ID, year and period showed that although there were significant differences between individuals ($F_{43,762} = 19.00$, $P < 0.0001$), and between years ($F_{15,762} = 5.39$, $P < 0.0001$), the largest effect was of period ($F_{2,762} = 121.31$, $P < 0.0001$). The effect of period was greater than the effect of month when the analysis was repeated with month instead of period ($F_{11,932} = 45.9$, $P < 0.0001$)

				regression				year		period		
Animal ID		Total time				Slope						
		(years)	n	Adj r^2	F	(kg.y^{-1})	P	F	P	F	P	n*
M	400B4C2E00	17.05	34	0.051	1.725	0.218	0.198	2.111	0.094	8.56	0.002	27
M	2223766B0A	15.11	43	0.036	2.561	0.242	0.117	1.668	0.137	9.461	0.001	35
M	404F1B1478	14.8	14	0.018	1.233	-0.305	0.289	6.975	0.039	12.738	0.001	14
F	405D2E4057	14.52	10	-0.125	0	-0.006	0.986	7.267	0.066	13.998	0.016	7
F	4059480F24	14.5	38	-0.027	0.042	0.034	0.839	4.349	0.002	2.405	0.108	32
F	22234D3237	14.37	7	0.347	4.184	-0.675	0.096	44.391	0.113	37.082	0.008	6
F	2223605D5E	14.34	66	-0.015	0.059	0.03	0.809	1.8	0.077	19.969	0.000	48
F	2223502753	13.95	31	0.018	1.555	0.226	0.222	3.446	0.01	9.214	0.001	26
F	405D1F4C64	13.21	11	-0.092	0.155	0.13	0.703	5.288	0.046	23.393	0.002	9
M	400B2C6D18	12.74	30	-0.023	0.357	0.112	0.555	1.911	0.153	2.159	0.137	27
M	412E7D0435	12.21	37	-0.028	0.033	0.03	0.858	3.726	0.009	4.189	0.026	30
M	400D361E7C	11.9	34	0.031	2.066	0.246	0.16	1.658	0.152	0.681	0.167	30
F	414B3F0118	11.34	14	0.505	14.241	0.737	0.003	5.785	0.015	16.19	0.001	12
M	413B56436C	10.67	8	0.584	10.843	-0.802	0.017	71.504	0.001	1.294	0.307	7
F	40597D0416	10.04	16	-0.043	0.383	-0.163	0.383	1.492	0.271	21.084	0.000	12

F	404F28052D	9.79	21	0.382	13.348	-0.642	0.002	4.711	0.008	3.076	0.081	16
F	400C2E2202	9.66	13	-0.086	0.052	0.069	0.823	10.085	0.004	1.439	0.3	10
M	412E392162	9.47	14	0.301	6.592	-0.595	0.025	4.68	0.027	13.841	0.001	14
M	413A7E4819	9.47	14	0.044	1.603	0.343	0.23	3.213	0.07	1.789	0.222	12
F	2223471B61	9.37	12	0.082	1.983	0.407	0.189	1.952	0.198	0.445	0.656	11
M	403A121970	9.05	8	-0.099	0.369	-0.642	0.566	3.151	0.148	1.346	0.298	7
F	404E7E0301	8.99	15	-0.052	0.307	0.152	0.589	1.178	0.403	12.569	0.005	13
M	403C621B75	8.78	86	0.014	2.23	0.161	0.139	6.387	0	9.494	0.000	71
M	412B326D5B	8.58	13	-0.079	0.12	0.104	0.735	0.916	0.431	0.156	0.858	13
F	401E045270	8.32	20	-0.027	0.5	0.164	0.488	5.68	0.004	8.887	0.004	15
F	405948006F	8.24	18	0.041	1.726	0.312	0.207	1.069	0.446	2.496	0.124	15
F	405C253A61	7.8	18	0.001	1.02	-0.245	0.328	1.006	0.468	8.203	0.004	18
F	400C193929	7.76	33	0.011	1.34	0.204	0.256	4.871	0.002	7.666	0.003	24
F	40611B792F	7.07	12	0.086	2.028	-0.411	0.185	1.186	0.395	1.611	0.266	10
M	40612C6C6C	6.96	18	-0.038	0.385	0.153	0.544	2.258	0.115	1.773	0.238	10
M	40611F4E53	6.72	14	-0.059	0.273	0.149	0.611	0.865	0.491	2.549	0.127	13
M	444D087A59	4.96	32	-0.003	0.892	0.17	0.352	0.945	0.432	7.576	0.003	26
F	2233445406	4.69	11	-0.087	0.197	-0.146	0.668	1.588	0.276	2.067	0.189	11
F	4061384815	4.65	55	-0.006	0.684	0.113	0.412	1.577	0.195	11.351	0.000	44
M	404E662517	4.43	17	0.073	2.251	0.361	0.154	6.368	0.005	9.459	0.005	13
F	413E7A6416	4.03	15	0.15	3.474	-0.459	0.085	0.727	0.593	7.644	0.008	14
F	4059167179	3.89	10	-0.054	0.538	0.251	0.484	0.578	0.586	1.77	0.232	8
F	405D337A7B	3.79	10	0.142	2.484	-0.487	0.154	0.368	0.779	17.829	0.003	10
M	4453595115	3.54	33	0.089	4.13	0.343	0.051	4.042	0.016	2.599	0.094	28
F	40613B6865	3.52	35	-0.027	0.093	-0.053	0.763	0.175	0.912	11.045	0.000	25
M	40612E4F1F	2.6	17	0.084	2.284	0.387	0.155	2.773	0.092	16.215	0.001	13
M	4059412601	2.01	11	0.522	11.92	0.755	0.007	4.235	0.056	0.47	0.51	11
F	4059165059	1.94	12	-0.074	0.244	0.154	0.632	1.124	0.367	1.437	0.261	11



Appendix – Figure 1. Graph showing the marked, consistent changes in relative adiposity (mass%) of 43 individual echidnas (19 males, 24 females) throughout the year. Blue squares represent males and red circles represent females. Grey bars denote the periods of prehibernation (January, February), reproduction (May – August) and post-reproduction (September – November). As data from individual echidnas from different years were treated as being statistically independent, there was up to 109 males and 150 females per month.

Chapter 3

The diet of the short beaked echidna (Tachyglossus aculeatus) assessed by analysis of faecal scats and of stable isotopes in blood

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Abstract

The short-beaked echidna (*Tachyglossus aculeatus*) is generally considered to be myrmecophagous, consuming a diet consisting of ants and termites. The range and seasonal variation of food items consumed by echidnas in the Southern Midlands of Tasmania, an area where termites are absent, was examined using faecal scat analysis and stable isotope analysis (ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$) of blood and potential prey items. Scat analysis indicated that Tasmanian echidnas are not purely myrmecophagous. Whilst ants were present in 97% of scats and made up 66 +36% of scat contents by % volume, non ant food items (cockchafer larvae and Oxycanus larvae) were found in 66% of scats. Pasture cockchafer larvae were present in 62% of scats, and in those scats made up 51 + 31% of the volume of prey material. The only significant seasonal variation of any dietary item was for cockchafer larvae, which can be attributed to variations in the abundance of these larvae throughout its lifecycle. The patterns of consumption indicate that echidnas are opportunistic foragers. Whilst the type of vegetation in which the core area of individual echidnas was located did not have any effect on scat contents, it did have a significant effect on the isotopic signatures of the blood of echidnas. Values of $\delta^{15}N$ indicated that echidnas inhabiting pasture habitats were feeding at greater trophic levels then echidnas inhabiting woodland or mixed habitats, in spite of scat analysis indicating they were consuming similar items. When the $\delta^{15}N$ and $\delta^{13}C$ of the ants within different habitats were determined, it was apparent that the isotopic signatures of echidna blood are due to differences in the isotopic composition of ants from different habitats, rather than from the consumption of different species in each habitat type. These results highlight the importance of habitat type in comparative SIA studies, and indicate that any isotopic variation between different habitat types must be considered.

Introduction

Ants and termites make up a large proportion of the world's biomass (Wilson and Hölldobler 2005). For example, over 25% of total animal biomass in the Amazonian rainforest comprises ants (Fittkau and Klinge 1973), and thus ants represent a large potential food source. Twenty two species of mammal are specialist ant and/or termite eaters (myrmecophages) (Redford 1987; Redford and Dorea 1984), and these include representatives of all three mammalian groups: monotremes, marsupials and eutherians (McNab 1984). Specialised myrmecophages show a number of anatomical adaptations including reduced or absent teeth and modifications of jaw and cranial musculature and the tongue (Reiss 2001) which enable rapid transfer of prey, minimising the defensive

behaviours of prey items (Redford 1987). The muscular tongue of myrmecophages is long and slender, allowing it to be rapidly protruded and retracted, while large salivary glands provide lubrication and help to pick up prey items (Griffiths 1968).

Although ants and termites may be very abundant, there are disadvantages associated with a myrmecophagous diet. Ants and termites have a low energy density due to their low digestibility (Redford and Dorea 1984). Their exoskeleton is primarily composed of chitin, which is an indigestible, or at best poorly digested, complex structural polysaccharide, with ants having proportionately more chitin then termites (Redford and Dorea 1984). Colony composition may be highly seasonal, with more nutritionally valuable pupae and larvae being produced in warmer months (Thomas 2003). Many species have highly developed cooperative defensive strategies, including biting, stinging and chemical secretion and alarm pheromones (Redford and Dorea 1984), which effectively reduce the amount of time a predator can spend foraging at a particular site. Finally, the irregular distribution of ant and termite nests further reduces their energy density, making them a resource that is patchy both in space and time (McNab 1984). Presumably because of the low energy density of their food, ant and termite eating mammals have low body temperatures and low rates of metabolism (McNab 1984; McNab 2000).

The short-beaked echidna (*Tachyglossus aculeatus*) and the numbat (*Myrmecobius fasciatus*) are the only Australian specialist mammalian myrmecophages. The numbat diet consists almost exclusively of termites and numbats are now restricted to the arid regions of south-west Western Australia (Friend 2008). By contrast, the short beaked echidna, *Tachyglossus aculeatus*, has a very wide distribution - it is found in all habitats across Australia, and in coastal and highland areas of New Guinea. Geographic variation in diet was addressed by Griffiths (1978) who examined faeces from animals from eight localities across Australia and found animals from areas of low annual rainfall and high summer temperatures consumed predominantly termites, whereas echidnas in cooler, wetter habitats consumed predominantly ants. A range of studies support this general pattern: echidnas in the temperate areas of New South Wales are primarily ant-eaters although they also consume some termites (Griffiths 1968; Smith *et al.* 1989). Echidnas in the Western Australian wheat belt consume a greater number of termites than ants (Abensperg-Traun and De Boer 1992; Abensperg-Traun 1988), whilst echidnas in

the tropical Kakadu National Park in the Northern Territory consume equal proportions of ants and termites (Griffiths *et al.* 1990). Along with a count of 15,000 ants, only 15 individual arthropods other than ants or termites were found in the Kakadu samples (Griffiths *et al.* 1990). All these studies concluded that echidnas appear to be opportunistic foragers – prey items are ingested in proportion to the amount encountered, although none of these studies actually measured food availability. This preference for termites in arid areas is not due to the absence of ants, and is found in lizards and some other mammals (Abensperg-Traun 1994). Griffiths (1989) and Abensperg-Traun (1988) suggest that in arid areas this preference for termites by echidnas is possibly due to the higher water content of termites (up to 80%) compared to ants (64% water).

Studies in the New England Tablelands and West Australian wheat belt found a substantial proportion of ants ingested were *Iridomyrmex* (Abensperg-Traun *et al.* 1991; Abensperg-Traun 1988; Falkenstein *et al.* 2001), and in temperate Victoria the species most commonly found in scats were *Rhytidoponera, Pheidole, Iridomyrmex* and *Myrmecia* (Harrison 1997). The most common dietary items apart from ants and termites are scarab larvae: in the New England region up to 37% of the average food biomass consisted of scarab beetle larvae (*Sericesthis* spp.) (Smith *et al.* 1989). Echidnas also exhibit seasonal changes in diet preference and foraging activity (Abensperg-Traun and De Boer 1992; Smith *et al.* 1989), most likely reflecting variation in the availability of prey items. The amount of scarab beetle larvae consumed varied seasonally. In New England maximum consumption of scarab beetle larvae occurred in summer when of 20% of diet consisted of larvae with none were consumed in winter (Smith *et al.* 1989). In north-eastern Victoria scarab beetle larvae were consumed throughout the year, with maximum consumption between July and September (Harrison 1997). Griffiths and Simpson (1966) also found echidnas in the ACT were strictly seasonal in their foraging for meat ants (*Iridomyrmex purpureus* formerly *I. detectus*). They observed echidnas attacking meat ant nests from late July into October; activity which ceased once the winged virgin queens and adult males left the nest for the nuptial flight.

Throughout Australia, echidnas are a highly seasonal species with a strong annual cycle in their behaviour and consequently feeding intensity varies greatly during the year (Green *et al.* 1992; Nicol and Andersen 2007a). During winter all echidnas reduce their foraging activity, and may enter hibernation or periods of torpor (Abensperg-Traun and

De Boer 1992; Beard *et al.* 1992; Green *et al.* 1992; Grigg *et al.* 1992; Rismiller and McKelvey 1996; Smith *et al.* 1989). The pattern of hibernation in echidnas varies between populations, duration decreasing with decreasing latitude and increasing altitude, with populations in Tasmania and Mount Kosciusko (NSW) hibernating for longer than other populations studied (Beard *et al.* 1992; Grigg *et al.* 1992; Nicol and Andersen 2002). Populations on Kangaroo Island, South Australia, have a greatly reduced hibernation period and exhibit a small number of brief bouts of torpor (Rismiller and McKelvey 1996). Rather than low temperatures or food shortages triggering hibernation, echidnas voluntarily reduce foraging and enter hibernation during late summer/autumn when temperatures are still mild and sufficient food available (Nicol and Andersen 2002).

As might be expected from their highly seasonal variation in activity, Tasmanian echidnas show similar seasonal variation in the intensity of their foraging. Adult male echidnas cease foraging and hibernate from mid February to early July, and adult female echidnas in reproductive years hibernate from mid March to late July, non reproductive females hibernating from mid March to late September (Nicol and Andersen 2007a). The reproductive period occurs almost immediately after males exit hibernation, when female echidnas are still hibernating (Morrow and Nicol 2009). Male echidnas forage intermittently during mating, but do not begin to replenish fat stores depleted during hibernation and mating until the end of the reproductive season (Nicol and Andersen 2007a). Female echidnas enter a nursery burrow within three weeks of arousal from hibernation, where they remain for up to six weeks nursing their young (Morrow *et al.* 2009). Exit from the nursery burrow initiates intensive feeding for reproductive females. In addition to obtaining sufficient calories for successful lactation, these females need sufficient food to replenish energy stores lost during hibernation and the time spent in the nursery burrow.

Some hibernating mammals change their diet before entering hibernation to maximise the intake of polyunsaturated fats to maintain cell membrane fluidity at low body temperatures (Florant *et al.* 1993; Geiser *et al.* 1992; Hill and Florant 2000; Munro and Thomas 2004; Munro *et al.* 2005). Falkenstein *et al.* (2001) found no evidence for this in echidnas in the New England region, but because Tasmanian echidnas are so strongly seasonal, it is possible that they may seek out specific dietary items.

The termite assemblage in Tasmania is highly reduced compared to mainland Australia. The three genera present (*Prototermes, Kalotermes* and *Stolotermes*) form small to moderate sized colonies within damp wood (http://anic.ento.csiro.au/database/biota_details.aspx?BiotaID=24884; Watson and Gay 1991) rather than the large nests found on mainland Australia. As a result the termite biomass available to Tasmanian echidnas is much lower than that available to mainland echidnas and they are unlikely to form an important source of energy. Based on twelve scats collected from the north-east of the state, (Griffiths 1978) estimated that the ratio of ants to termites consumed was 98% ants and 2% termites. Termites were present in scats and gut contents of road killed animals and were also eaten by a free ranging, tame echidna, along with a range of invertebrates, including ants, the larvae and/or adults of beetles, moths, flies, crickets, slaters, millipedes and spiders (Spencer and Richards 2009). However in their study Spencer and Richards (2009) only noted the presence of prey items and did not carry out any quantitative analysis.

As Tasmanian echidnas have a shorter active period than other populations of echidnas and a shorter time to restore their energy stores after reproduction and lactation a major aim of this study was to investigate whether the strong seasonality of their life history is reflected in their diet. Is there any evidence of termites being eaten at specific times of the year, and if not, how does the absence of termites affect the diet? What is the range of dietary items consumed and do they differ between different habitats? To investigate these questions, two techniques were used: scat analysis and stable isotope analysis (SIA). Scat analysis provides information on actual dietary items consumed through the identification of undigested fragments of invertebrates in the faeces, so actual diet and any fine scale variation in dietary items consumed can be determined. The basis underlying the use of stable isotope in dietary studies is that the isotopic rations of carbon ^{13}C:^{12}C and nitrogen^{15}N:^{14}N in the tissues of dietary items are transferred to the body tissues of the animals that consume them, in a predictable and reliable manner. Heavier isotopes form bonds of greater energy than their isotopically lighter counterparts and thus are less likely to undergo chemical reactions. The lighter isotope is preferentially excreted by the consumer, and the heavier one retained, so the tissues of the consumer become enriched relative to their diet (DeNiro and Epstein 1978; DeNiro and Epstein 1981; Hobson *et al.* 1993; Hobson and Clark 1993). The fractionation of ^{15}N from ^{14}N in

animals occurs during amino acid synthesis, which results in the retention of isotopically heavier ^{15}N and the excretion of the isotopically lighter ^{15}N. ^{15}N accumulates rapidly and can be used to assess the trophic position of organisms in food webs with enrichment averaging 3 – 5‰ at each trophic level (DeNiro and Epstein 1981; Minagawa and Wada 1984; Peterson and Fry 1987). In contrast, limited enrichment (0-1‰) of ^{13}C occurs with each trophic exchange (DeNiro and Epstein 1978), instead ratios of ^{13}C ($\delta^{13}C$) in organisms reflect that of their diet and can be used to determine the principal sources of carbon for a consumer when the isotopic signatures of the sources are different (Peterson and Fry 1987). Coupled $\delta^{13}C$ and $\delta^{15}N$ measurements thus provide trophic and source information.

Materials and Methods

Animal capture, scat and blood collection

Fieldwork was carried out at Lovely Banks, a grazing property in the Southern Midlands, 55 km north of Hobart, Tasmania (longitude 147 14', latitude 42 25'S). Vegetation at the site consists of remnant dry sclerophyll woodland dominated by *Eucalyptus amygdalina* interspersed with improved and native pasture and patches of *Acacia dealbata*, *Casuarina verticilata* and *Lomandra longifolia*. The site is intersected by numerous gullies, caves and sandstone outcrops and ranges in altitude from 200 to 400 m asl. Highest temperatures are recorded in January (mean maximum 23.9°C, mean minimum 10.3°C), and the lowest in July (mean maximum 11.4°C, mean minimum 2.0°C). Annual mean rainfall is 447 mm which is spread throughout the year, with highest falls in the period August-November (http://www.bom.gov.au/climate/averages/tables/cw_094201.shtml).

Up to 12 adult echidnas in any year had a radio transmitter (Bio-telemetry Tracking, St Agnes, South Australia) glued to the spines on the lower back using 2-part epoxy glue so they could be located. All individuals captured at the site had a passive transponder tag (PIT, LifeChip; Destron Fearing) injected subcutaneously, allowing identification upon capture. When each animal was located, a blood sample was taken from the rostral sinus under light isoflurane anaesthesia. Approximately 1 ml of blood was drawn into a syringe and placed on ice until arrival at the laboratory. Blood samples were collected approximately every two months throughout the year. Echidnas would

normally defecate when handled and these faecal samples were collected opportunistically and either stored in alcohol or frozen for future analysis.

A total of 8 species of ants were collected from the study site to form a reference collection, and additional information for identification of the remaining four species was obtained by examination of the B. B. Lowery Collection held at Forestry Tasmania, Hobart a reference collection containing all ant species known to occur within Tasmania, and discussion with P. McQuillan (Department of Geography and Environmental Studies, University of Tasmania). Samples of 4 common ant species were collected from woodland and pasture habitat during spring to enable comparison of isotopic composition of ants from these habitats. As scat analysis indicated that the larval stages of blackheaded pasture cockchafers are ingested, two cockchafer larvae (*Acrossidius tasmaniae*) were also collected from pasture for stable isotope analysis.

Laboratory procedures

Blood samples were centrifuged at 6000 rpm for approximately 10 minutes, gently stirred to break up any fibrin clots and centrifuged for a further 10 minutes. The serum was then drawn off with a pipette and used for hormone analysis (see Chapter 2), and the red blood cells stored at -20^0C for stable isotope analysis.

Frozen faecal samples were thawed, mixed with water and gently broken up. Samples stored in alcohol normally broke up during storage. Material was washed through a sieve stack (75 μm, 150μm, 300 μm, 600 μm and 1.18mm) to remove the soil and sort the prey contents by size. The content of each sieve was examined under a dissecting microscope. Although ant prey are highly fragmented in the digestive tract the head capsules remain intact, and these were used to identify ants down to genus using the reference specimens. There were no heads in the smallest two sieves, so material that fell within these size classes was discarded. Because of the range of sizes of ants within some genera, head capsules from the same genus were identified in more than one sieve. An absolute count of ant heads within each size category within each genus was made for each scat sample.

A number of prey types could not be identified down to family including the alate forms of ants, which were assigned to the general group ‘alates’; ants that could not be identified and were only were occasionally observed were placed in the group ‘other’. These groups were treated as taxa during statistical analysis. Head capsules and

mandibles of pasture cockchafers were used to quantify numbers of larvae ingested. If mandibles were present in a scat they were counted and the number divided by two to get the minimum number of larvae, and head capsules were ignored; if no mandibles were present the number of head capsules was counted.

To provide a semi-quantitative estimation of the relative contribution to the diet of each prey category a correction factor was applied to items from each sieve size. Ants from the smallest size sieve (300μm) were given a value of 1; those from the 600μm sieve a value of 2, and ants from the largest sieve (1180μm) were assigned a value of 4. These corrected values were then summed for each ant genus to give a measure of the volume contributed by that dietary item. The relative contribution to the diet of each prey category within an individual scat sample was then calculated as a percentage of the total corrected number of head capsules within the scat.

The relative contribution of pasture cockchafers was calculated assuming one pasture grub equalled approximately 270 ants from a 300 μm sieve, based on the mass of a whole single large cockchafer larvae and the mass of an individual ant of genus *Pheidole*. To allow comparison with other studies, scats were also scored for the presence or absence of specific dietary items. Following Redford (1987), this presence/absence data is referred to as *percent occurrence*, whereas the relative contribution is referred to as *percent volume*. To investigate whether there predominance of a specific ant size, ants were classed as large (head capsules retained in 1.18 mm sieve), medium (head capsules retained in 600 μm sieve) and small (head capsules retained in 300 μm sieve), and the percentage of each ant size group calculated as a percentage of the total number of ants counted in the scat.

Stable isotope analysis

Red blood cell samples were thawed and dried at 80^{o}C for 24 hours. Between 10 – 25 individuals of *Rhytidoponera sp., Pheidole sp.,* and *Camponotus sp.* were collected from woodland and pasture habitat during spring, however, in spite of intensive searching *Anonychomyrma sp*. could only be found in woodland. Invertebrate samples were prepared by removing the caudal portion of the abdomen of pasture grubs and the gaster of ant specimens to eliminate the influence of undigested food on isotopic analysis (Bluthgen *et al.* 2003). Invertebrate samples were oven dried at 80°C and then ground

using a mortar and pestle. All samples were sent to the Australian National University Stable Isotope Laboratory for analysis.

Stable isotope abundances were expressed in delta notation as deviations from standards in parts per thousand (‰) and calculated using the following equation:

$$\delta X = [(R_{sample} / R_{standard}) - 1] \times 1000$$

where δX is ^{13}C or ^{15}N and R is the corresponding ratio ^{13}C:^{12}C or ^{15}N:^{14}N. $R_{Standard}$ values were based on PeeDee Belemnite (^{13}C) and atmospheric air (^{15}N).

Calculating core home range and habitat type

For each individual echidna, core home range was estimated as described in Chapter 4. In brief, only those animals for which there were a minimum of 20 locations in any one year were used, excluding consecutive positions where the echidna was in the same location, due to hibernation or occupancy of a nursery burrow. The kernelUD function within the adehabitat R package (R Development Core Team 2009) was used to calculate the 50% (core) kernel of the home range of each individual echidna, using a grid size of 20m and a smoothing factor (h) of 50. Home range data were then imported into ArcMap 9.2, and the 50% kernel laid over a geo-referenced high resolution (QuickBird) image of the study site in which the position of woodland vegetation had been digitised. For each animal the predominant vegetation type in the animal's core area (50% kernel) was used to allocate the animal to one of three habitat categories: pasture, woodland, or a mix of pasture and woodland.

Statistical analysis

Scat analysis

For the percent occurrence data, differences between groups were investigated using Chi-squared tests. Because the percent volume data was a semi quantitative measure based on sieve size, differences between groups were investigated using the non-parametric Kruskal-Wallis analysis of ranks in the statistical package Statistica (Statistica 6.1, StatSoft, Tulsa, OK). Differences in diet between sexes, habitat (pasture, woodland, mixed) and season were tested separately. For seasonal analyses samples were allocated to one of three periods depending on collection date: prehibernation (January – March), reproduction (June – September), post-reproduction (October – December). When there was more than one scat sample for an individual animal in a given period, the values

were averaged. This resulted in no individual contributing more than one sample per period. Samples from other dates were not included in this analysis. These periods delineate quite different physiological states for echidnas (see Appendix to Chapter 2), which could be reflected in different feeding strategies. During prehibernation echidnas are preparing for entry into hibernation, energy stores are near maximal and feeding activity is declining. Reproduction occurs immediately after hibernation; females have a brief opportunity to feed before entering the nursery burrow, while many males may show brief periods of intense feeding, however body mass declines during the reproduction period. The post-reproductive period is the period of maximum foraging and mass gain. Differences in prey size between groups (sex, season and habitat type) were tested separately for each size class using Kruskal-Wallis analysis of ranks.

Stable isotopes

When there was more than one data point for an individual animal in a given period, a random number generator was used to randomly select one sample to include in the analysis. Carbon and nitrogen isotope data were both normally distributed (D'Agostino-Pearson omnibus normality test, carbon K2 = 1.34, P= 0.51; nitrogen K2 = 3.1, P = 0.21) and both data sets were analysed using one way ANOVA to test the effect of season, habitat and sex on $\delta^{13}C$ and $\delta^{15}N$ using the statistical package Statistica (Statistica 6.1, StatSoft, Tulsa, OK).

Results

Faecal Analysis

A total of 57 scats were collected from 29 individual echidnas (21 females and 8 males). When duplicate samples from individuals in a given period were averaged, 44 independent data points (referred to as scats for consistency) remained for statistical analysis. Of these samples 13 were from animals with core areas in bush (10 females, 3 males), 22 mixed (14 females, 8 males), and 9 in open areas (all females). Eleven samples were collected during the *prehibernation* period (9 females, 2 males), 8 during the *reproductive* period (4 females, 4 males), and 15 during the *post-reproductive* period (12 females, 3 males). A further 10 samples were collected outside of these periods. Two samples were found to consist almost entirely of what appeared to be the pupae of the *Oxycanus* grass grub (*Oxycanus australis*). All other scats contained highly fragmented

ants. The head, legs and gaster were usually detached from the mesosoma, and antennae detached from the head. Eleven ant genera were identifiable in the scats. Another clearly distinct species of *Dolichoderine* ant could not be identified to genus. A complete listing of ant genera is given in Table 1.

Table 1. Relative volumes of ant genera in echidna scats from Lovely Banks field site, based on 44 scats. A species resembling *Anonychomyrm*a was clearly distinguishable in the scats but was not in the reference collection, or identifiable in published literature, and is simply listed as "*Unidentified Dolichoderine*". Alates and cockchafer larvae have been excluded from the data. Information on type of defence mechanism is from http://anic.ento.csiro.au/ants/. Values shown are means ± SD.

Ant genus	Subfamily	% volume of ants in diet	Defence type
Anonychomyrma	*Dolichoderinae*	22.0 ± 18.4	Chemical
Pheidole	*Myrmicinae*	20.9 ± 15.8	Stinging
Notoncus	*Formicinae*	13.3 ± 17.0	Chemical
Rhytidoponera	*Ectatomminae*	9.7 ± 12.0	Stinging
Solenopsis	*Dolichoderinae*	8.9 ± 14.9	Stinging
Iridomyrmex	*Myrmicinae*	8.3 ± 8.3	Chemical
Ochetellus	*Dolichoderinae*	4.6 ± 12.1	Chemical
Camponotus	*Formicinae*	4.0 ± 5.9	Chemical
Unidentified Dolichoderine	*Dolichoderinae*	3.4 ± 6.6	Chemical
Other		2.6 ± 4.6	
Melophorus	*Formicinae*	2.1 ± 4.1	Chemical
Myrmecia	*Myrmeciinae*	0.2 ± 0.3	Stinging
Polyrhachis	*Formicinae*	0.1 ± 0.2	Chemical

Multiple unidentified ant egg cases were also present but it was not possible to quantify these as only fragments were present. Twenty-six scats contained head capsules or mandibles of the larval form of pasture cockchafer beetles belonging to the family Scarabaeidae. Five species of cockchafer larvae are known to occur at the field site (*Adophius tasmaniae, Adophius pseudotasmaniae, Adoryphorus couloni, Scitala sericans, and Sericesthis nigra*) (McQuillan *et al.* 2007), but it was not possible to identify these to species or even genus level from head capsules and mandibles only, and they have been grouped together as cockchafer larvae.

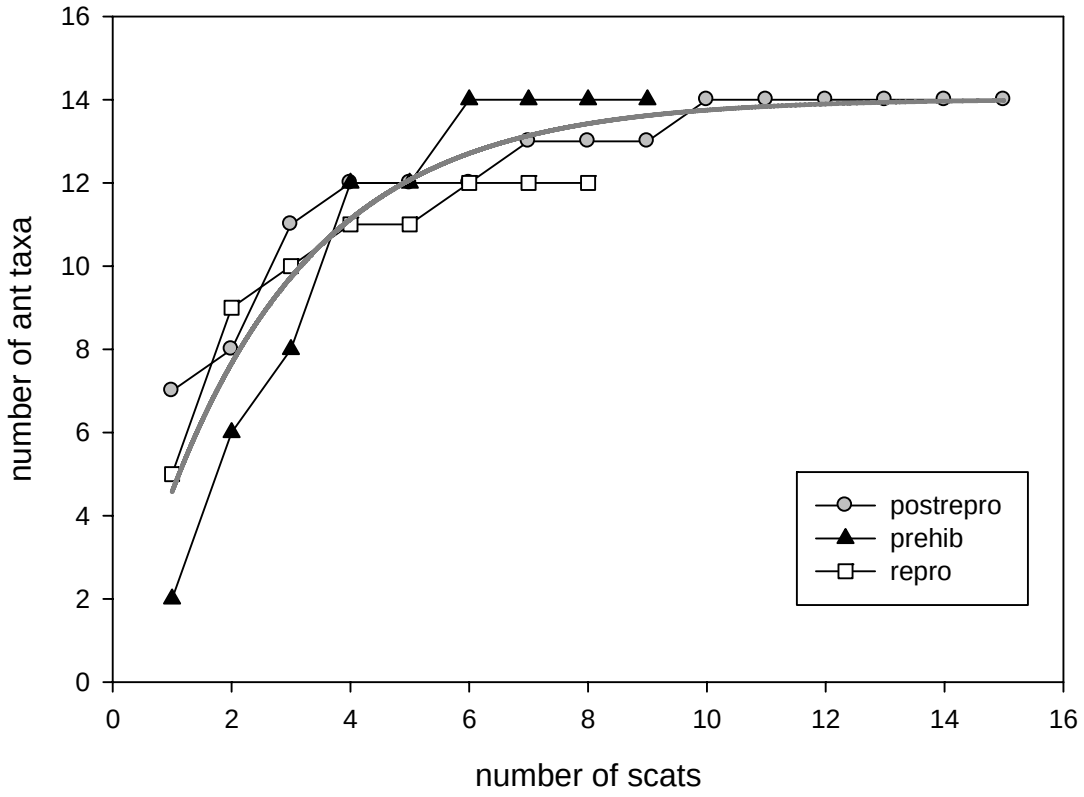

Figure 1. Number of ant taxa identified as a function of number of echidna scats examined. Scats were collected during the postreproductive period, prehibernation period and the reproductive period. For the purpose of this analysis, the categories "alates" and "other" have been treated as taxa.

The cumulative number of ant taxa reached an asymptote after analysis of 7 randomly selected scats (Fig. 1). Fig. 2 shows the percentage of scats containing specific prey items (percent occurrence). The two most common genera were *Pheidole* (present in 89% of scats) and *Rhytidoponera* (76% of scats). Six ant genera *Anonychomyrma, Pheidole, Rhytidoponera, Notoncus, Iridomyrmex and Solenopsis* made up 82% of the volume of ant material identified in the scats (Figure 3). Ants were found in 96.7% of scats and made up 66.5 $\pm$ 36.3% of scat contents by % volume. Food items other than ants (pasture cockchafer larvae and *Oxycanus* larvae) were found in 65.5% of scats. Pasture cockchafer larvae were found in 62% of scats (Fig 2), and in those scats they made up 50.9 ± 31.2% of the volume of prey material (range 4 – 98%).

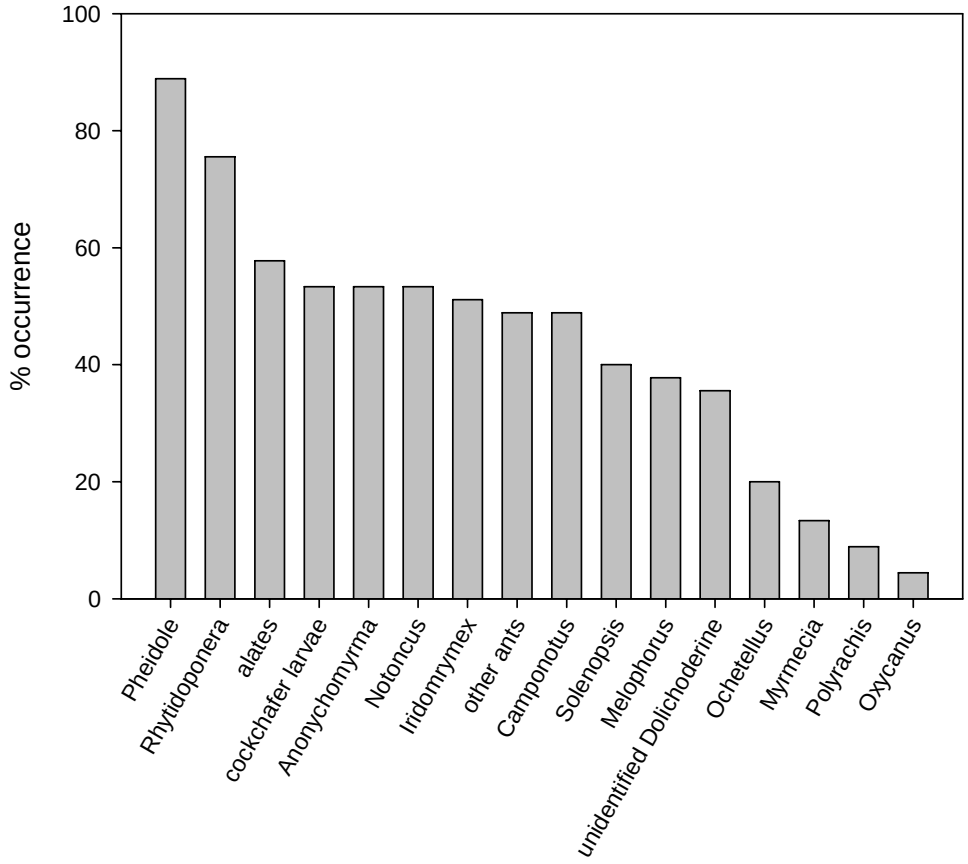

Figure 2. Percent occurrence of all prey items identified in 44 echidna scats. The bars show the percentage of scats which contained each prey item.

Overall, there was no significant difference in the % occurrence of dietary items in the scats of male and female echidnas ($\chi^2_1 = 0.21$, $P = 0.65$). The only significant seasonal variation in % occurrence was for cockchafer larvae ($\chi^2_2 = 15.6$, $P < 0.001$) which were only present in 27% of scats during the *prehibernation* period, while during the *reproduction* and *post-reproduction* periods they were present in 88% and 80% of scats respectively. There was no effect of habitat on the % occurrence of ants in scats.

There was a significant difference in the % volume of the ant genus *Pheidole* in the diets of male and female echidnas (Kruskal-Wallis test $H_{(1,29)} = 4.01$, $P < 0.05$), with a higher contribution of *Pheidole* to the diets of females (15.4 $\pm$ 3.4%, SE, n=21) than males (1.7 $\pm$ 5.5%, SE, n=8). There was also a significant difference in the % volume of cockchafer larvae in the diets of male and female echidnas (Kruskal-Wallis test $H_{(1,29)} = 4.75$, $P < 0.05$), with a higher contribution of larvae to the diets of males (65.0 $\pm$ 12.0%,

SE, n=8) than females (29.7 ± 7.4%, SE, n=21). Analysis of data for each period showed that the low value of cockchafer larvae was largely due to low consumption by females during the *reproduction* period (females 33.8 ± 14.6, n = 4; males 89.5 ± 14.6, n=4) (Kruskal-Wallis test $H_{(1,8)} = 4.13$, $P < 0.05$). Correspondingly, females had a higher consumption of *Pheidole* during *reproduction* period (females 9.7 ± 5.1, n = 4; males 0.7 ± 5.1, n=4) (Kruskal-Wallis test $H_{(1,8)} = 5.33$, $P < 0.05$).

Using the data set consisting of one value per period per animal, analysis of the effect of period on % volume of prey items showed a significant differences in the consumption of the unidentified Dolichoderine species (Kruskal-Wallis test $H_{(2,34)} = 8.32$, $P < 0.02$). Percent volume of this prey was higher in *post-reproduction* (0.89 ± 0.33) and *prehibernation* (0.62 ± 0.38) periods compared to the *reproductive* period (0.12 ± 0.45). There was a highly significant effect of period on the % volume of cockchafer larvae consumed (Kruskal-Wallis test $H_{(2,34)} = 10.0$, $P < 0.01$). During the *reproduction* period they contributed a mean of 60.15% ± 11.18 (n = 8), during the *post- reproduction* period 54.11% ± 8.12 (n = 15) and during the *prehibernation* period the amount that cockchafer larvae contributed to the diet dropped to 16.07% ± 9.53 (n = 11).

There was no effect of habitat on the % volume of all prey species using combined data for males and females in woodland and mixed habitats only (as males did not have home ranges located exclusively in pasture), and no effect of habitat on % volume of all prey species when female data from pasture, mixed and woodland habitats was analysed.

Echidnas ate significantly more individuals of small and medium sized ants than large sized ants (Kruskal-Wallis test $H_{(2,126)} = 56.77$, $P <0.0001$), with the number of large ant prey items being significantly lower than that of the other two size classes (multiple comparison *z'* values: large vs small = 6.95, large vs medium = 5.99, small vs medium 0.96). The mean percent volume of small sized ants was 34.0 ± 25.8% (SD, range 0 -95%), medium size ants 43.4 ± 22.2% (SD, range 2 – 84%) and for large sized ants 22.9 ± 19.3% (SD, range 0 – 75.0). There was no difference in distribution of the size of prey items between sexes (Kruskal-Wallis test small: $H_{(1,42)} = 1.97$, $P = 0.16$; medium: $H_{(1,42)} = 0.31$, $P = 0.58$; large: $H_{(1,42)} = 0.11$, $P = 0.74$), period (Kruskal-Wallis test small: $H_{(2,32)} = 0.87$, $P = 0.65$; medium: $H_{(2,32)} = 2.92$, $P = 0.23$; large: $H_{(2,32)} = 1.82$,

$P = 0.40$) or habitat types (Kruskal-Wallis test small: $H_{(1,33)} = 3.60$, $P = 0.17$; medium: $H_{(1,33)} = 0.62$, $P = 0.73$; large: $H_{(1,33)} = 1.46$, $P = 0.48$).

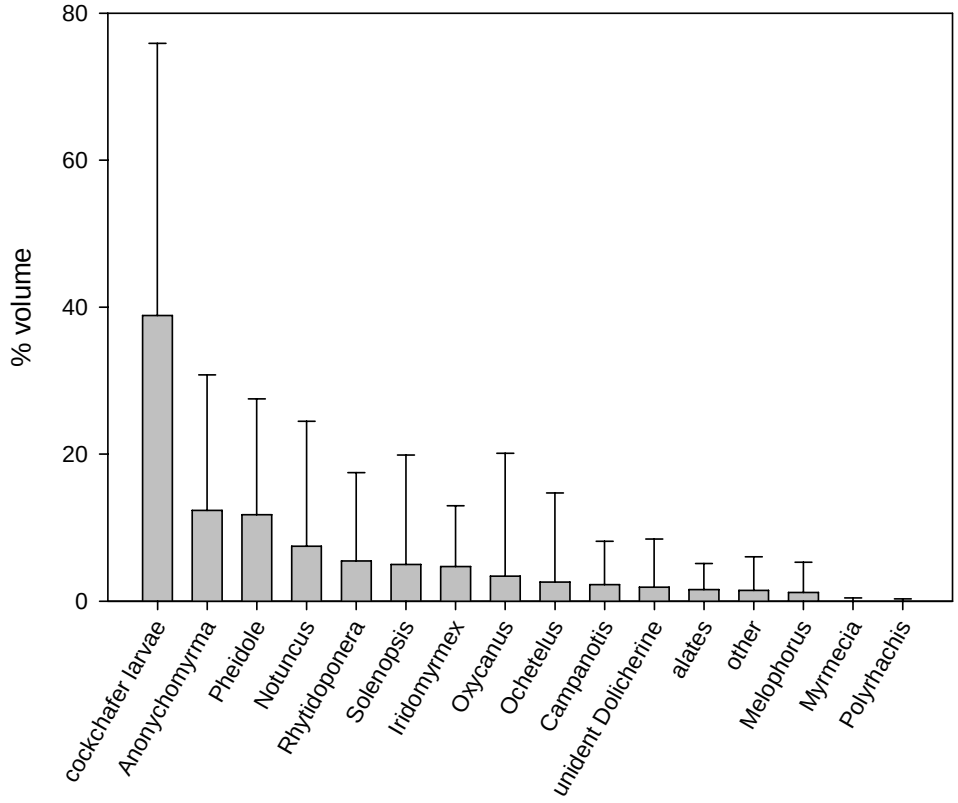

Figure 3. Percentage volume of diet by dietary item. The bars show the mean contributions of various prey items to the diet of the short beaked echidna, based on analysis of 45 scats. Error bars are standard deviations.

Stable isotope analysis

$\delta^{15}N$ and $\delta^{13}C$ signatures of echidnas

A total of 63 blood samples from 14 female and five male echidnas were analysed for stable isotopes. Where there were multiple samples from the same animal in a given period or habitat type, a random number generator was used to randomly select one sample to include in the analysis. These results are shown in figure 4. The $\delta^{13}C$ blood isotope signatures of echidnas ranged from -24.3‰ to -21.86‰, and $\delta^{15}N$ values ranged

from 3.9‰ to 9‰. These $\delta^{15}N$ values suggest that echidnas occupy a range of trophic positions.

Factorial ANOVA for sex and period (*prehibernation, reproduction, post-reproduction*) on the whole data set showed no effects of sex or period on carbon (sex: $F_{(1,21)} = 1.98$, $P = 0.17$; period: $F_{(1,21)} = 0.08$, $P = 0.92$). There was no effect of period on nitrogen ($F_{(2,21)} = 0.15$, $P = 0.87$) but there was a significant difference in nitrogen between sexes ($F_{(1,21)} = 5.7$, $P < 0.05$). As there were no males with exclusively open habitats the effect of sex and habitat was investigated by excluding data from animals from open habitats. There was no effect of sex on carbon ($F_{(1,15)} = 1.61$, $P = 0.22$) but habitat type (mixed and woodland) had a significant effect on carbon ($F_{(1,15)} = 5.27$, $P < 0.05$). For nitrogen there were significant effects of habitat ($F_{(1,15)} = 6.0$, $P < 0.05$) but no effect of sex ($F_{(1,15)} = 3.62$, $P = 0.07$). The lack of any effect of period was further shown by one-way ANOVA of data from one individual male echidna for which there was 20 samples (carbon: $F_{(2,14)} = 2.5$, $P = 0.12$; nitrogen: $F_{(2,14)} = 3.4$, $P = 0.06$). Only one sample per period from this animal was used in other analysis. The lack of significant variation in $\delta^{13}C$ indicates that the source of dietary carbon is the same for males and females and echidnas inhabiting different habitats. Values of $\delta^{15}N$ indicate that echidnas inhabiting pasture habitats are feeding at a greater trophic level then echidnas inhabiting woodland or mixed habitats.

$\delta^{15}N$ and $\delta^{13}C$ signatures of prey items

The result of stable isotope analysis of prey items is given in Figure 5, along with mean isotope ratios of echidna red blood cells to illustrate the isotopic position of prey items relative to habitat types in home ranges. The number of samples was too small for statistical analysis, but there appears to be a division of $\delta^{15}N$ between prey items collected in pasture and woodland. Prey items collected from pasture were much more enriched in $\delta^{15}N$ than those collected from woodland, with the exception of the ant species *Camponotus* and pasture cockchafer larvae. *Camponotus* had a low $\delta^{15}N$ value regardless of the habitat from which it was collected, and cockchafer larvae, which were collected from pasture habitat, also had very low $\delta^{15}N$, as well as being highly depleted in $\delta^{13}C$.

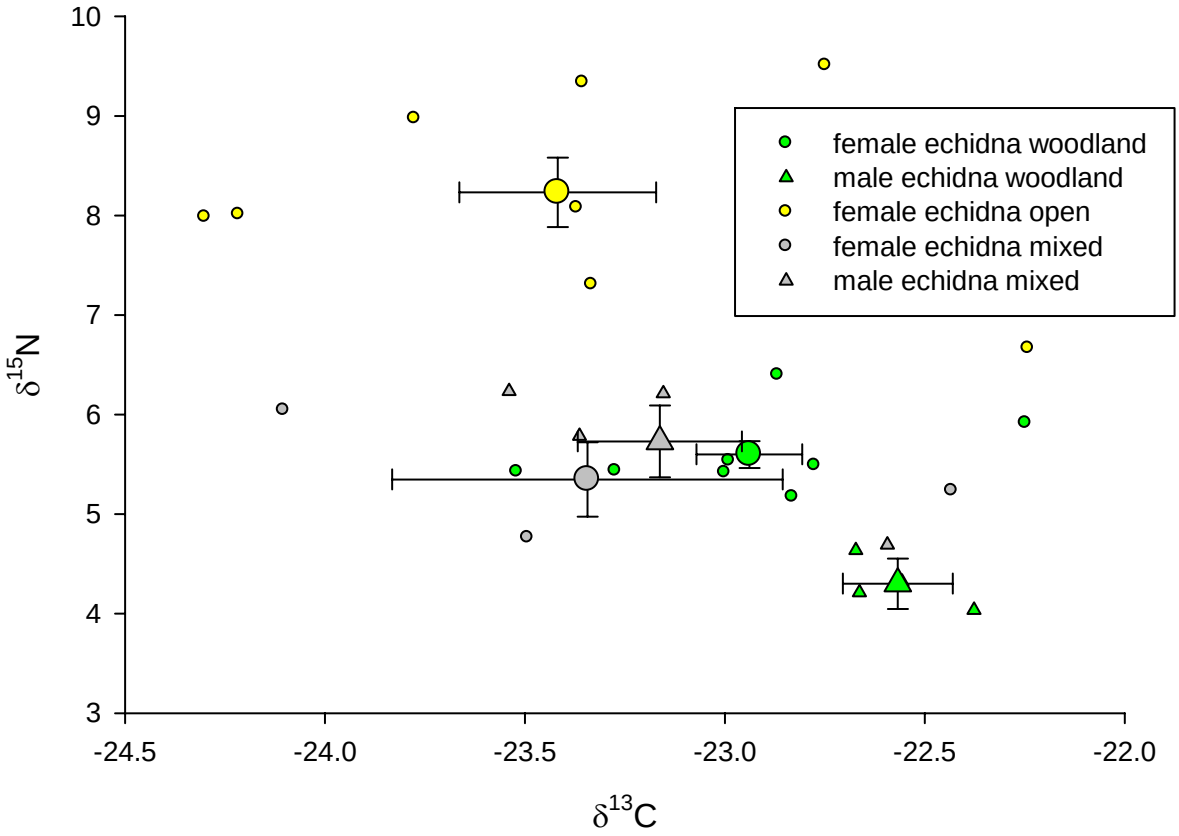

Figure 4: Stable isotope ratios of carbon and nitrogen of red blood cells of echidnas (*Tachyglossus aculeatus)*. Echidnas are grouped by the predominant vegetation type within their core home range. "Mixed" vegetation is a relatively even mix of woodland and pasture. There were no male echidnas with home ranges consisting of predominantly pasture. Large symbols are mean values for each sex within each vegetation type ± S.E.

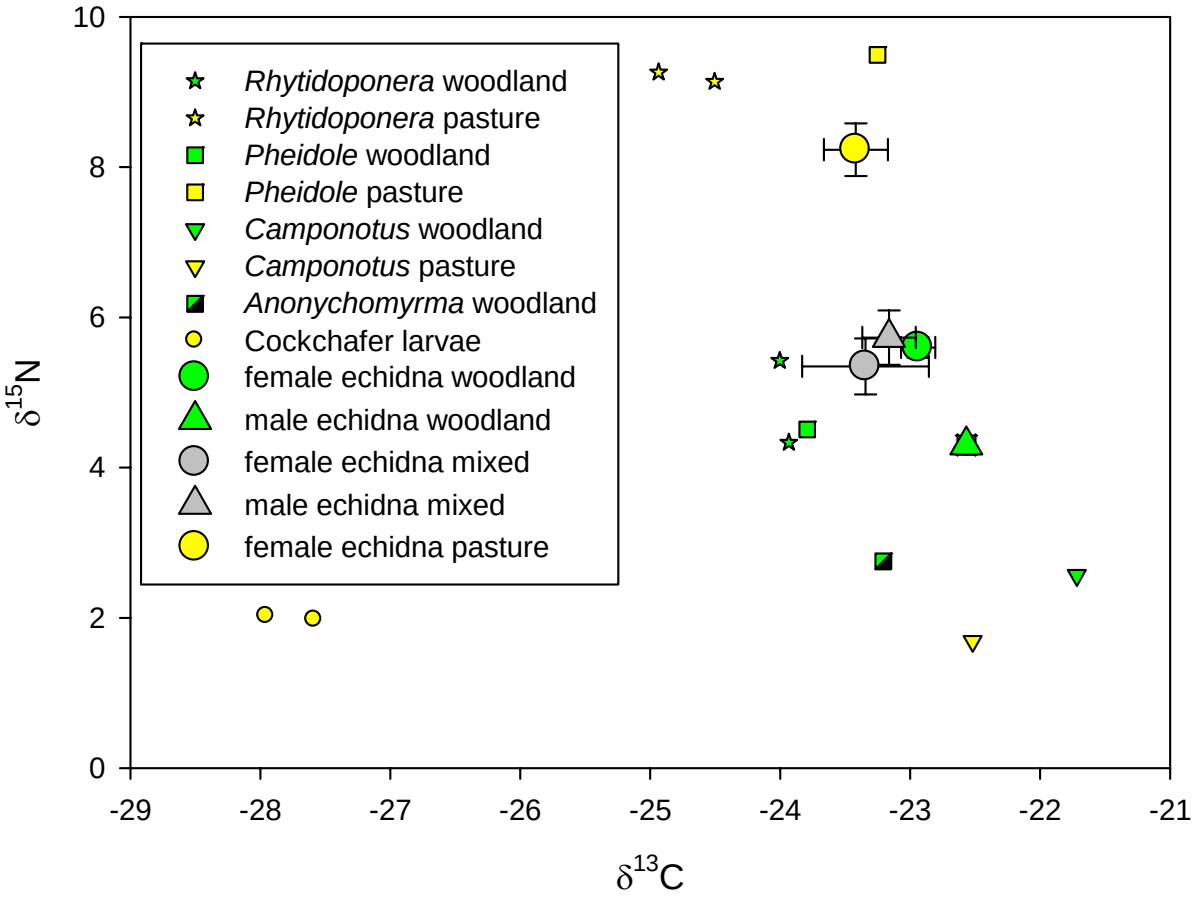

Figure 5. Stable isotope ratios of carbon and nitrogen in potential prey items (small symbols) and red blood cells of the echidna (*Tachyglossus aculeatus*) (large symbols). Samples were grouped by the vegetation type from which they were collected (prey) or by the predominant vegetation type within core home range (echidna blood samples). 'Mixed' refers to a relatively even mix of pasture and woodland within the home range. Values of $\delta^{15}N$ and $\delta^{13}C$ for echidna blood samples are mean $\pm$ standard error of the mean for each sex in each vegetation type.

Discussion

Compared to other scat analysis studies, only a relatively small number of echidna scats were examined. This precluded detailed analysis of the effects of season and habitat on the composition of prey species. However, it is clear from the species accumulation curve that the scats examined were reasonably representative of the diet of echidnas, and the overall results supported the main conclusion that the diet of echidnas at the Tasmanian midlands field site consisted almost entirely of ants and pasture grubs, and there was no evidence of termites in the diet. Approximately two thirds of the estimated food volume was ants, the other third comprised the underground larvae of cockchafer beetles or moths. Of the ant species consumed, six genera *Anonychomyrma, Pheidole, Rhytidoponera, Notoncus, Iridomyrmex and Solenopsis* accounted for over 80% of ant content of scats overall, with relatively few large sized ants being eaten. One of the principal reasons for this study was to investigate whether the strong seasonality of echidna behaviour and physiology was reflected in seasonal variations in their diet. The only dietary components that showed a significant seasonal effect were cockchafer larvae and unidentified Dolichoderine ants. Although the variation in % volume of the unidentified Dolichoderine was statistically significant, the contribution of these ants to the diet of echidnas was so minor (0.12 – 0.89%), they are unlikely to be biologically important. In contrast, cockchafer larvae contribute a large percentage to the diet of echidnas (16 – 60%). The low percent occurrence of cockchafer larvae in echidna scats during the *prehibernation* period reflects a much lower abundance in the soil during the period January – March (McQuillan *et al.* 2007). There was no effect of habitat on the % volume of ants in scats. The absence of any habitat effect is in contrast to the results of stable isotope analysis: there were no seasonal effects on carbon or nitrogen isotope ratios, but there were very significant habitat effects.

Diet composition

The ant prey consumed by echidnas are summarised in Table 1. The five subfamilies consumed are characterized by different defence mechanisms: as is the case for other anteaters (Redford 1987), none of these mechanisms seems more or less effective in deterring echidnas from eating small and medium sized ants. However, large aggressive

ants of genus *Myrmecia* (bull ants and jack jumpers) are rare dietary items, although they are a species commonly encountered at Lovely Banks. The more effective defensive behaviours of larger ant species may negate any energetic advantage to echidnas foraging on larger prey. Almost 90% of scats contained *Pheidole* (Fig. 2), but this species only contributed 12% by volume to the diet (Fig. 3). This indicates that although widespread throughout the site and commonly encountered and consumed by foraging echidnas, because of their small size they do not contribute a large volume to the diet. Although Myrmecinae such as *Pheidole* are capable of stinging and biting simultaneously (Redford 1987), the small size of *Pheidole* may limit the effectiveness of their defence against foraging echidnas. *Rhytidoponera* were also present in a large proportion of scats, but like *Pheidole*, contributed only a relatively small volume to total scat content (5%). *Rhytidoponera* nest in soil and would frequently be encountered by foraging echidnas and their large size would make them a valuable prey item. However, the relatively large size of these ants probably makes their aggressive defensive behaviour an effective deterrent for echidnas, limiting the duration of foraging in the nests of this species. *Anonychomyrma* was present in one quarter of all scats and contributed 12% to total diet by volume. *Anonychomyrma* are an important diet species for echidnas – they form moderate to large nests (http://anic.ento.csiro.au/ants/biota_details.aspx? BiotaID=35540; Shattuck 1999) and are likely to be frequently encountered by foraging echidnas as they were frequently observed whilst undertaking field work. Dolichoderinae such as *Anonychomyrma* lack a functional sting, relying on the release of chemicals for defence. This defence mechanism appears to have limited effect on echidnas as large numbers of this prey are consumed, with 22% of ant prey consisting of *Anonychomyrma*.

At mainland sites, echidnas consume large numbers of termites in areas where they are highly abundant (Griffiths 1978, Abensperg-Traun & De Boer 1992, Abensperg-Traun 1988) and diet may consist of up to 94% termites (Abensperg-Traun and De Boer 1992). There was no evidence of termites in scat samples collected from Lovely Banks echidnas, and no termites were found at the field site in spite of searching for them. Where present, Tasmanian termites live in relatively small colonies and construct simple nests consisting of a series of chambers and galleries excavated in wood (Watson and Gay 1991) and would be unlikely to form a major part of the diet of Tasmanian echidnas due to their low abundance. The higher proportion of indigestible chitin results in ants

being lower in nutrition than termites (Redford and Dorea 1984), and in areas where termites are absent or in low abundance it appears that echidnas supplement their diet with nutritionally valuable non-ant prey. In areas of mainland Australia where termites are abundant, there is a low incidence of ingestion of prey other than ants or termites, with no other dietary items recorded for the WA wheatbelt (Abensperg-Traun and De Boer 1992) and only 15 non-ant or termite items encountered in 170 scats in the Northern Territory (Griffiths *et al.* 1990). In temperate areas, including the current study site in the Tasmanian midlands, echidnas appear to compensate for this absence of termites by increasing their consumption of non-ant prey, particularly, the larvae of beetle species (Smith *et al.* 1989).

Seasonality

There was little variation in ant species consumed throughout the year. Although ant availability could not be determined, the lack of seasonal variation in ant species consumed (percent occurrence in scats) indicates that all ant species are probably available for predation throughout the year. The absence of any biologically relevant seasonal variation in scat composition by volume is consistent with the suggestion that echidnas adopt consistent foraging behaviours and forage opportunistically throughout the year. This opportunistic pattern of predation is also adopted by other myrmecophages, including aardwolves (*Proteles cristata*), tamandua anteaters (*Tamandua sp*.) and giant anteaters (*Myrmecophaga tridactyla*), which vary the intake of prey species their diet depending on their availability (Kruuk and Sands 1972; Montgomery and Lubin 1977; Redford 1985).

The pattern of consumption of pasture cockchafer larvae also suggests that echidnas are opportunistic foragers. Mean contribution to diet was lowest during the *prehibernation* period (summer) when numbers of larvae are lowest. The two most common species black headed cockchafer larvae (*Acrossidus tasmaniae* and *A. pseudotasmaniae*) have a one year lifecycle. Adult beetles emerge during mid-late summer, mate and lay eggs which develop into fully grown larvae by late autumn, which then develop into pre-pupa and pupa in the prehibernation period in early summer. The less common *Adoryphorus couloni* has a two year lifecycle (McQuillan *et al.* 2007). Adults of the red headed cockchafer larvae *Adoryphorus couloni* emerge in late winter, mate and lay eggs which develop into fully grown larvae in late spring. They remain in

this larval stage for 10 months before developing into pre-pupa and pupa in the prehibernation period in early summer (McQuillan *et al.* 2007). The different life cycles of the red and black headed cockchafer larvae means that larvae are available for predation by echidnas throughout the year, but numbers are lower during summer *prehibernation* period. There was no evidence of consumption of pupal or adult stages, as presumably they are unpalatable or inedible to echidnas once they pass from the soft larval stages into sclerotised pupae and adult beetles, and the reduction in abundance of larvae in scats during *prehibernation* likely reflects the reduced availability of cockchafer larvae during this period. The seasonal pattern of consumption in Tasmania was similar to that of echidnas living in north-eastern Victoria (Harrison 1997). However, echidnas living on the New England Tablelands (NSW) show an opposite pattern of consumption of cockchafer larvae to Tasmanian echidnas; and were absent from scats collected in winter, but occurred in 20% of scats during summer, contributing an estimated 37% of the average food biomass intake (Smith *et al.* 1989).

Habitat

The type of vegetation in which the core area of individual echidnas was located did not have any effect on scat contents, but had a dramatic influence on the isotopic signatures of the blood of echidnas. Individuals living in predominantly open pasture areas had enriched $\delta^{15}N$, those living in woodland habitats were significantly less enriched in $\delta^{15}N$, and echidnas whose core area encompassed both woodland and pasture had low to moderate enrichment of $\delta^{15}N$ – slightly greater than the N signature of woodland animals. This result highlights a limitation of using only SIA in diet studies, as it could be interpreted as indicating that echidnas from different habitats are consuming different food items. However, when the $\delta^{15}N$ and $\delta^{13}C$ of the ants within different habitats were determined, it was apparent that the isotopic signatures of echidna blood are due to differences in the isotopic composition of ants from different habitats, rather than from the consumption of different species in each habitat type.

Two ant species collected from open pasture were more enriched in $\delta^{15}N$ than the same species collected from woodland, however *Camponotus sp.* collected in open pasture habitat was highly depleted in $\delta^{15}N$ which was similar to the sample collected from a woodland habitat. The low $\delta^{15}N$ of *Camponotus* is consistent with low values of *Camponotus* obtained by Gibb and Cunningham (2011) from similar habitats in SE

Australia, and shows that this species relies more on a plant based diet rather than predation. Gibb and Cunningham (2011) found that other ant species shift their trophic position under differing land management regimes, with ant assemblages in pasture feeding higher in the food chain than those feeding in revegetated sites, a pattern that is in accordance with my results, albeit at a much larger geographical scale than our study site. These results indicate that ant genera such as *Rhytidoponera* and *Pheidole*, which are general predators and scavengers (http://anic.ento.csiro.au/ants/), have a plant based diet in woodland habitats and are more predatory in pasture areas.

Insectivores have significantly lower $\delta^{15}N$ enrichment than consumers of other high protein diets. Instead of the generally predictable increase between 2‰ and 4‰ at each trophic level (DeNiro and Epstein 1981; Fry 2006), a relatively low enrichment ranging from 1.03‰ to 1.4‰ from insect prey to insectivorous consumer occurs (Herrera *et al.* 2001; McCutchan *et al.* 2003; Tillberg and Breed 2004). However, the magnitude of change between woodland and pasture dwelling echidnas (~3‰) was similar to that shown by predators and their non-insectivorous prey in other studies (DeNiro and Epstein 1981). Animals living in pasture and woodland habitats are consuming the same items, as evidenced by scat analysis, but the trophic level at which some of these dietary items are foraging differs, and this appears as a full trophic level difference between echidnas foraging in different habitats.

Sex differences

The differences in the % volume of cockchafer larvae in male and female scats were confined to the *reproductive* period. This is the period when the activity of males and females is very different. Movement, and presumably foraging, by females is limited as if they are active and not still hibernating, they are usually being pursued by males. Males however are ranging widely to locate females. This difference in cockchafer larvae in scats during this period may be a reflection of the difference in the ranging behaviour of male and female echidnas.

Differences in stable isotopes between males and females may reflect the very strong effect of vegetation type on isotopic signatures, and the fact that males have much larger home ranges (Chapter 4 and Nicol *et al.* 2011). The vegetation at Lovely Banks consists of a mosaic of woodland and pasture. The home ranges of males in woodland vegetation, although consisting predominantly of woodland, will by virtue of their larger

size, also include some patches of pasture. The inclusion of some pasture habitat should result in the blood of woodland males having $\delta^{15}N$ enrichment more akin to that of animals inhabiting mixed habitat, instead of the depleted $\delta^{15}N$ observed. It may be that these males are foraging in areas within the woodland that female echidnas do not use.

Limitations of scat analysis and problems of assessing food availability

Trites and Joy (2005) have addressed the problem of sample size in scat analysis, but as shown in Fig. 1, the cumulative number of ant taxa reached an asymptote after analysis of 7 randomly selected scats, indicating that an adequate number of scat samples were analysed to describe the diet of echidnas. Although the numbers of scats used to compare ants consumed in different habitats and seasons were low, rather than examine absolute contributions of ant species to the diet of echidnas, the main aims of this study was to determine the types of prey consumed by Tasmanian echidnas, and the relative contribution of ant and non-ant prey in their diet in the absence of termites. Scat analysis showed the range of ant genera that were consumed by echidnas (% occurrence) and provided an indication of the relative importance of different ants in terms of diet composition.

The main limitation of scat analysis is differential digestion of dietary items, leading to the underestimation of easily digested prey (Dickman and Huang 1988). Thus high quality food items, which are also most digestible, such as ant eggs and pupae and soft bodied invertebrates, may not be observable in the scats and are certainly not quantifiable. The eggs and pupae of ants were frequently observed in scats, however their highly fragmented state meant calculating consumption was impossible.

The lack of any seasonal variation in diet, with the exception of pasture cockchafer larvae, whose dietary abundance reflects seasonal variation in numbers in the soil, suggests that echidnas eat what is available. However the problems associated with measuring the abundance of ants make this difficult to assess. It was not practicable to conduct an assessment of prey availability. Abundance of ants is normally assessed by use of baited traps and pitfalls. However these methods only reflect invertebrate activity at the surface rather than being a measure of abundance (Greenslade 1964). Echidnas do not eat individual surface active ants but feed from subterranean ant colonies, sometimes exposing them using the claws on the front feet, but often just by pushing the beak into the ground or under rocks. Counting numbers of surface foraging ants can underestimate

populations greatly as up to 80% of ants remain within the nest and do not participate in foraging behaviours (Ayre 1962). A comparison of pitfall traps and mapping ant nests has also found that species recorded as rare from pitfall traps had the densest nests (Debruyn 1993). Thus pitfall traps are not a valid method of assessing prey availability for echidnas. The soil at the field site, whilst relatively sandy, was very rocky, making it impossible to dig up nests in order to determine abundance and availability of ants. When it was possible to dig up sites where echidnas had been feeding there was usually no indication at all as to what had been predated.

Although a strong preference for prey items may mask seasonal availability, there is no way to determine diet preference in echidnas. Diet choice experiments on captive animals have not yet been successful for echidnas as free ranging echidnas brought into captivity generally do not eat. Similarly, determining relative abundance of key resources of prey species is difficult as little research on ants and cockchafer larvae has been carried out in Tasmania, so what these resources might consist of is unknown.

Comparison of scat analysis and stable isotopes as techniques for dietary analysis

There was no indication that pasture cockchafers influenced the isotope signatures of echidna blood. $\delta^{15}N$ and $\delta^{13}C$ in echidna blood were much more closely aligned with the isotopic signatures of ant prey (Figs. 4 and 5). This was surprising given the predominance of pasture cockchafer larvae in the scats. This may be due to the temporal differences between the passage of scats and assimilation of stable isotopes of dietary items into the red cells. Scats reflect the food consumed by an echidna approximately two days prior (Griffiths 1965) whereas the isotopic signature of echidna red cells will reflect food items consumed in the previous four to six weeks (Andersen *et al.* 2000). During hibernation, red cell turnover is decreased (Andersen *et al.* 2000) which could further confuse the picture. However, it is hard to see how this could explain the absence of any isotopic evidence of ingestion of cockchafer larvae. The cockchafer larvae analysed were collected from pasture areas, where they feed on the roots of grasses, and subsequent to this analysis, pasture cockchafer larvae have been found in significant numbers in woodland. Analysis of stable isotopes of larvae from woodland habitats may reveal a signature that is more aligned to echidna blood signatures, and the current results could

indicate that echidnas do not consume cockchafer larvae within pasture habitats, but forage for them in woodland.

Stable isotope analysis has emphasised the importance of spatial aspects of the foraging ecology of echidnas rather than attempting to trace the elements through the ecosystem. These results highlight the importance of habitat type in comparative SIA studies, and indicate that future studies need to take into account any isotopic variation between different habitat types.

Conclusion

The Tasmanian echidna consumed predominantly ants but also large amounts of the larvae of pasture cockchafer beetle species. Despite their strong seasonal variation in activity the only seasonal variation found in the echidna diet was for pasture cockchafer larvae, which can be attributed to variations in the abundance of larvae throughout its lifecycle. During the prehibernation period, when pasture cockchafer larvae are less abundant (McQuillan *et al.* 2007), the presence of several scats containing only the larval stages of *Oxycanus* grass grubs suggests that echidnas seek out and possibly require appropriate non-ant dietary items throughout the year.

The morphology of the feeding apparatus of the echidna suggests that it is at the extreme end of specialisation of feeding on ants and termites (Reiss 2001). Hence, it would be expected that, like other mammals with extreme morphological specialisation for myrmecophagy, echidnas would feed exclusively on ants and/or termites, as appears to be the case for ground pangolins *Manis temminckii* (Swart *et al.* 1999), and the tamandua *Tamandua sp*. (Lubin and Montgomery 1981; Oyarzun *et al.* 1996). However, it is clear that an apparent extreme adaptation to a myrmecophagous diet does not prevent the echidna from supplementing an ant diet with soft-bodied soil invertebrates where termites are not available. As has been demonstrated for the nine banded armadillo (*Dasypus novemcinctus*), anatomical specialisation does not preclude dietary variation (Smith and Redford 1990), and this flexibility provides some explanation of why the echidna is so widespread in Australasia.

Chapter 4

The influence of habitat type on the home range size of the short beaked echidna (*Tachyglossus aculeatus*)

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Abstract

The size of an animal's home range is strongly influenced by the resources available within it. In productive, resource rich habitats sufficient resources are obtainable within a smaller area, and for many species, home ranges are smaller in resource rich habitats than those in habitats with lower resource abundance. Location data on 14 male and 27 female echidnas (*Tachyglossus aculeatus*) fitted with tracking transmitters, in the southern midlands of Tasmania, were used to test the influence of habitat type on home range size. I hypothesised that as woodland offered more shelter, potential refuges and potentially more food resources than pasture, echidnas living in woodland would have smaller home ranges than those living in pasture areas. Results indicated significant differences between the sexes. Male echidnas had a significantly larger mean home range (KH90 61.2 $\pm$ 20.5 ha, core 18.7 $\pm$ 5.9 ha) than females (KH90 34.5 $\pm$ 13 ha, core 11.2 $\pm$ 4.4 ha), and a quite different relationship between home range size and habitat type than females. There was no relationship between the proportion of woodland within male home range and home range size whereas female echidnas had a highly significant negative relationship. This suggests that home range size of female echidnas is highly influenced by the amount of woodland within it, but the home range size of male echidnas is controlled by factors other than habitat. This pattern is consistent with the spatial ecology of many other solitary species with a promiscuous mating system. The home ranges of females are generally scaled to encompass all necessary resources for successfully raising their young within a minimal area, whilst the large home ranges of males are scaled to maximise access to females.

Introduction

Understanding the factors determining the spatial distribution, resource utilisation and movements of animals within their home range is critical when considering species ecology. Home range is defined as the area in which an individual carries out its normal activities of foraging and reproductive behaviours (Burt 1943), and must encompass sufficient resources to fulfil the animal's energetic requirements. An animal's home range normally extends beyond providing an adequate food supply and includes requirements such as shelter and potential mates, and consequently must accommodate all required

resources across the entire year. The availability and distribution of these factors will influence the shape and size of an animal's home range, and there may be marked temporal shifts in home range use in response to seasonal changes in the availability of resources, including potential mates (Dahle and Swenson 2003b; Stirrat 2003). A hierarchical pattern in the factors affecting home range size has been suggested, with three different levels: the species level; the population level and the individual level (McLoughlin and Ferguson 2000).

Factors that influence home range size at the species level include average body size, trophic level and climate (McLoughlin and Ferguson 2000). The best single predictor of home range size is body size, accounting for more than 70% of the variation between species (Calder 1984). Like other metabolically based parameters, home range size (*H*) scales with body mass according to the allometric relationship

$$H = aM^b$$

where M = body mass and a is a coefficient which may vary between trophic groups. The exponent (*b)* ranges from just under 1 to 1.9 which is surprisingly high for a metabolic parameter (McNab 2002). In their extensive review of home range sizes of 279 species of eutherian mammal, (Kelt and Van Vuren 2001) found similar exponents for carnivores, omnivores and herbivores (1.13 for the pooled data) but different values of the coefficient (*a*) for each trophic group, so that carnivores have larger home ranges than similar sized omnivores, which in turn have larger home ranges than herbivores (Kelt and Van Vuren 2001). These patterns may be explained not only by the relative abundances of the foods for each trophic group, but also by their relative metabolic rates (McNab 2002).

At the population level, food availability and habitat productivity have been suggested as primary determinants of the home range size of different populations of a species (McLoughlin and Ferguson 2000). For some species, home range size may be relatively constant, with population density being the main population parameter that varies with food availability (Makarieva *et al.* 2005).

At the individual level, patchiness of resources, density and sex all play a role in determining home range size, but primarily it is resource availability that drives the size of individual home ranges (McLoughlin and Ferguson 2000). The amount of edge habitat is also known to be important in determining home range size in some species (Bixler

and Gittleman 2000; Ries and Sisk 2004; Saïd and Servanty 2005). A home range must be large enough to contain sufficient resources, but not so large that it prevents familiarity of the area for the occupant (McLoughlin and Ferguson 2000).

Ant- and termite-eating animals show significant deviation from these general patterns. The data on other myrmecophagous species (Heath and Coulson 1997; Joshi *et al.* 1995; Medri and Mourão 2005; Skinner and Van Aarde 1986; Taylor *et al.* 2002) suggests that home range does not scale with body mass in this trophic group in the same way it does for other groups, with the exponent (*b*) in the allometric equation being only 0.61 and not significantly different from zero (Nicol *et al.* 2011). Ant-eating mammals have a lower basal metabolic rate compared to other mammals, which falls further below the predicted level as their body size increases (McNab 2000). These low energy requirements, and apparent lack of relationship between home range size and body mass, suggest that the relationship between home range size and resource availability may differ between ant-eating species and other trophic groups.

At the species level, the myrmecophagous short beaked echidna (*Tachyglossus aculeatus*) has a smaller home range than comparably sized eutherian carnivores and omnivores (Nicol *et al.* 2011). Population densities of echidnas from various parts of Australia may vary more than home range size (Nicol *et al.* 2011), but there are insufficient comparative data to undertake a detailed comparison and investigate the influence of factors such as environmental patchiness, climate and ecosystem productivity. In the Tasmanian echidna study population, male home ranges are significantly larger than female home ranges; annual home range size (90% kernel) for males is 107 ± 47 ha (SD), range 35-164 ha, for females 48 ± 28 ha, range 17- 104 ha, and there is no correlation between body mass and home range size for either sex (Nicol *et al.* 2011). Visual inspection of the data from that previous study (Nicol *et al.* 2011) suggests that, for female echidnas at least, home ranges in areas that are predominantly pasture are larger than those in woodland. In many species, differential utilisation of resources through habitat partitioning may occur between conspecific individuals of the opposite sex. For example for both deer mice (*Peromyscus maniculatus*) and mountain pygmy possums (*Burramys parvus*), females occupy small home ranges in good quality habitats, and males are confined to the more marginal habitats, only entering the good quality habitats during mating seasons (Bowers and Smith 1979; Mansergh and Scotts

1986), and male otters and mink use large streams more frequently than females, who predominantly use smaller streams and lakes (Kruuk 1995; Reid *et al.* 1994). The differences in home range sizes of female echidnas in different habitat types may be due to differential utilisation of resources in those habitats.

In this chapter I investigate the relationship between home range size and vegetation type, including the interface between two vegetation types (edge habitat). The study site consists of a mosaic of improved and native pasture and woodland. Since woodland contains much more shelter and potential refuges such as hollow logs, and is likely to have more food resources, it is likely to represent higher quality habitat than pasture. At this site, the predominant food items of echidnas are ants and the underground larvae of scarab beetles (Chapter 3). Although the primarily myrmecophagous echidna forages on prey that is widespread and abundant, the distribution of ant nests and scarab larvae is likely to be dependant on vegetation. Consisting of a grassy understorey, bracken fern, *Acacia* shrubs and trees and tall eucalyptus tree canopies, and fallen logs and coarse woody debris, woodland at the study site provides a higher degree of structural complexity than pasture. Localised patches of *Lomandra* tussocks occur within both woodland and pasture, and there are some isolated large eucalyptus trees (both dead and living) in pasture, but generally pasture has few other non-grass species within it. Abensperg-Traun and De Boer (1992) found a foraging preference in woodland and mallee habitats over shrub land and heath, indicating that some habitat types are likely to be more valuable to echidnas.

The high degree of structural complexity in woodland also offers a range of sheltering opportunities for echidnas. Echidnas use shelters extensively for protection from predators and reducing the energetic costs of thermoregulation (pers. obs.; Bennett 1881; Semon 1899; Smith *et al.* 1989; Wilkinson *et al.* 1998). In warmer regions, echidnas are active when air temperature ranges from 9° to 32°C (Abensperg-Traun and De Boer 1992; Augee *et al.* 1975; Augee *et al.* 1970), and retreat to shelters once air temperatures fall outside of this range. At high altitudes, and in the cool temperature and temperate regions of their range, echidnas use shelter sites more extensively and hibernate for up to 8 months of the year (Beard and Grigg 2000; Beard *et al.* 1992; Grigg *et al.* 1992; Nicol and Andersen 2007a).

We hypothesised that animals in areas with less woodland cover would have larger home ranges. Nicol *et al.* (2011) suggested that female home ranges are determined by resources, while males have larger home ranges to maximise mating success, a pattern that is seen in a range of solitary carnivores (Clutton-Brock 1989; Sandell 1989). Consequently, the relationship between home range size and woodland cover should differ between the sexes. As in the case of other mammal species, edge has been shown to have an influence on the home range size of bridled nailtail wallabies *(Onychogalea fraenata)* (Fisher 2000) , roe deer (*Capreolus capreolus*) (Saïd and Servanty 2005), and pumas (*Puma concolor)* (Laundré and Loxterman 2007), and it may be possible is that echidna home range size is dependent on available edge habitat.

Methods

Capture of animals

Fieldwork was carried out at Lovely Banks, a grazing property in the Southern Midlands, 55 km north of Hobart, Tasmania (longitude 147 14', latitude 42 25'S). Vegetation at the site consists of remnant dry sclerophyll woodland dominated by *Eucalyptus amygdalina* interspersed with improved and native pasture and patches of *Acacia dealbata*, *Casuarina verticilata* and *Lomandra longifolia*. The site is intersected with numerous gullies, caves and sandstone outcrops and ranges in altitude from 200 to 400 m asl.

Up to 12 echidnas in any one year (1997-2009) had a small radio transmitter (Bio-Telemetry Tracking, St Ages, South Australia) glued to the spines of the lower back using 2-part epoxy glue. Transmitters weighed 20g which is less than 1% of total body mass of an adult echidna. Animals were monitored one to two times a week. They were initially tracked from a vehicle equipped with a roof mounted whip antenna, and then located on foot using a hand held yagi antenna once a sufficiently strong signal detected (Sirtrack Ltd, Havelock North, New Zealand). Once an animal was located its position was recorded using a hand held GPS unit (Garmin International Inc., Kansas, USA). Any animals observed whilst driving around the site were caught, sexed, weighed and scanned for the presence of a subcutaneous passive transponder tag (LifeChip, Destron Fearing, MN, USA) using a hand held RFID reader (Destron Fearing, MN, USA). Individuals without a tag were tagged under light isoflurane anaesthetic.

Home range and habitat analysis

Annual home ranges of individual echidnas were estimated essentially as described in Nicol *et al.* (2011), except that less smoothing was applied to the data which gives weighting to areas of higher use (Worton 1989). In brief, animals were only included if they had a minimum of 20 locations, with a relatively even distribution throughout the year to avoid seasonal bias of location. This number was deemed appropriate to provide unbiased estimates of echidna home range by Nicol *et al.* (2011) who used an incremental analysis (Kernohan *et al.* 2001) based on the assumption that home range estimates reach an asymptote with adequate sample size (McLoughlin and Ferguson 2000). Although there were gaps in the series of location data, due to high home range fidelity of echidnas (Nicol *et al.*, 2011) this was not considered to be important.

Consecutive positions where the echidna was in the same location, due to hibernation or occupancy of a nursery burrow were excluded. Data used by Nicol *et al.* (2011) was also used plus some additional locations and animals. The mean number of points used to construct individual home ranges was 53 and ranged from 21 to 164 points. The 50% (core area, KH50) and 90% (home range, KH90) kernel of the home range were calculated for each individual echidna using the kernelUD function within the adehabitat R package (R Development Core Team 2009) and then imported into ArcMap 9.2 using a grid size of 20m and a smoothing factor (h) of 50. These kernels were then laid over a geo-referenced QuickBird image of the study site in which the position of woodland vegetation had been digitised, and for each kernel the total area and the proportion of pasture and woodland was calculated. Despite the high resolution of the image (0.6 m), it was not possible to differentiate pasture from *Lomandra* tussocks and areas of native grasses, so all areas that were not woodland were classified as pasture. The QuickBird image was captured in February 2007. Comparisons with aerial photographs captured over the previous 20 years indicated that any changes in vegetation margins during the course of the study were relatively minor, and it was taken to be a good representation of the vegetation in the study area.

To test whether the interface between the bush and pasture (edge) was an important factor in determining the size of home ranges, a 20 m buffer was constructed either side of the interface between bush and pasture using ArcGIS. Twenty metres was

chosen as this has been shown to be the maximum extent that trees at the woodland edge influence microhabitat, through leaf litter, seed dispersal, and effect of shading/light on soil microclimate (Reyes-López *et al.* 2003; Williams-Linera 1990). The area of this 'edge' habitat was determined for each home range.

Statistical Analysis

Statistical analyses were performed using the software package Statistica 6.1 (Statsoft, Tulsa, Oklahoma). Data were tested for normality using the Shapiro-Wilks W test; non-normal data sets were compared using the Mann-Whitney U Test, and log-transformed for regression analysis. Regressions were compared using the GLM heterogeneity of slopes procedure in Statistica. Unless otherwise specified averages are presented throughout as mean ± SD.

Results

Home ranges areas (KH90) and core areas (KH50) were calculated for 14 male and 27 female echidnas. For males, both parameters were normally distributed (KH90 Shapiro-Wilk W = 0.911, P = 0.162; KH50 Shapiro-Wilk W = 0.934, P = 0.345) with mean KH90 61.2 ± 20.5 ha (range 31.1 – 101.2, median 57.5) and mean core area 18.7 ± 5.9 ha (range 10.0 – 30.0, median 17.2). For females, neither parameter was normally distributed (KH90 Shapiro-Wilk W = 0.877, P < 0.01; KH50 Shapiro-Wilk W = 0.873, P < 0.001), mean KH90 was 34.5 ± 13.0 ha (range 19.2 -70.3, median 29.2) and KH50 was 11.2 ± 4.4 ha (range 5.8 – 23.8, median 9.7). Males had significantly larger KH90 and KH50 than females (KH90: Mann-Whitney U = 48, $n_1 = 27$, $n_2 = 14$, P < 0.0001; KH50: Mann-Whitney U = 56, $n_1 = 27$, $n_2 = 14$, P < 0.0001). Data sets for KH90 and KH50 were log transformed to normalise them for statistical testing.

Home range size and vegetation type

There was a significant difference between males and females in the slopes of the regressions of woodland area within the home range (KH90) against log KH90 (ANCOVA: $F = 18.54$; d.f. = 1,37; $P < 0.001$, Fig 1a), and for the regressions of woodland area within the core area (KH50) against log KH50 (ANCOVA: $F = 27.55$; d.f. = 1,37; $P < 0.0001$, Fig 1b). For males there was a strong positive correlation between the area of woodland within the home range and core area and the home range and core area size. For females there was no correlation between the area of woodland within the home

range and core area and home range and core area size. The slope and intercepts of these regressions are summarised in Table 1. The mean area of woodland in the core area (KH50) of female echidnas was 4.32 ± 2.47 ha, and in home range (KH90) it was 13.31 ± 7.33 ha.

When the area of woodland in the home range and core areas was expressed as a proportion of the total area there was no relationship for males (Fig. 2a and 2b) but for females, home range and core size decreased with increasing proportion of woodland (Figure 2a and 2b). The slope and intercepts of these regressions are summarised in Table 1.

Table 1: Results of the analysis of the relationship equations between size of home range (KH90), core home range (KH50) (Figure 1) of the short-beaked echidna (*Tachyglossus aculeatus*), and total woodland area and proportion of the total area of KH90 and KH50 that is woodland vegetation (Figure 2).

Total woodland area		Slope	Intercept	r^2	*P*
males					
	KH90	77.79	-4137	0.51	< 0.005
	KH50	30.48	-148.6	0.63	< 0.001
females					
	KH90	- 1.13	75.96	0.05	0.25
	KH50	+ 3.96	24.27	0.06	0.20
Proportion of woodland					
males					
	KH90	- 0.12	1.70	0.02	0.59
	KH50	+ 0.21	-0.48	0.02	0.63
females					
	KH90	– 1.21	7.12	0.44	< 0.001
	KH50	– 1.3	7.01	0.46	< 0.0001

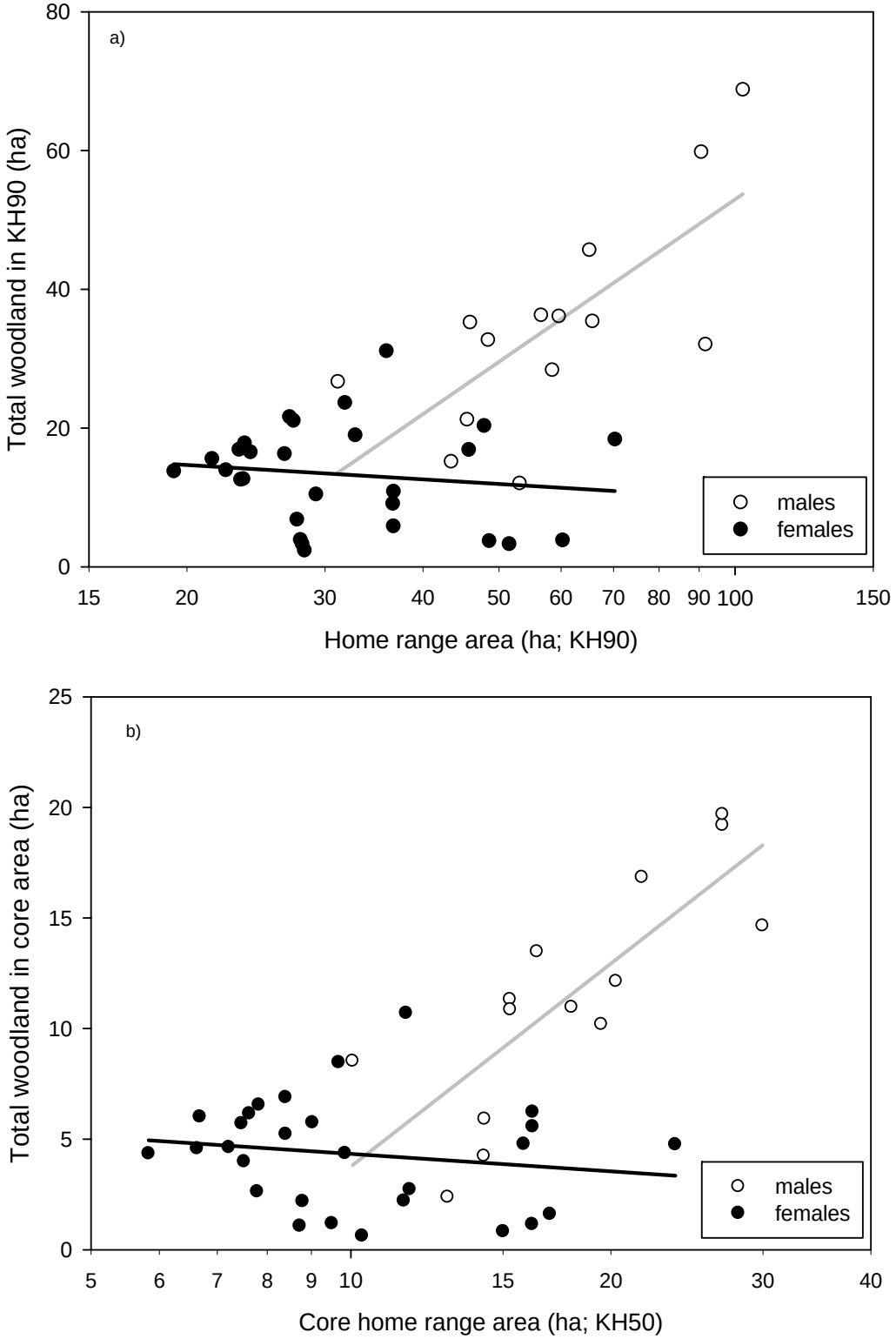

Figure 1. The relationship between the size of a) KH90 and b) core and area of woodland vegetation within the home ranges of male and female echidnas *(Tachyglossus aculeatus)*. Values of home ranges and core areas are log-transformed to meet assumptions of normality.

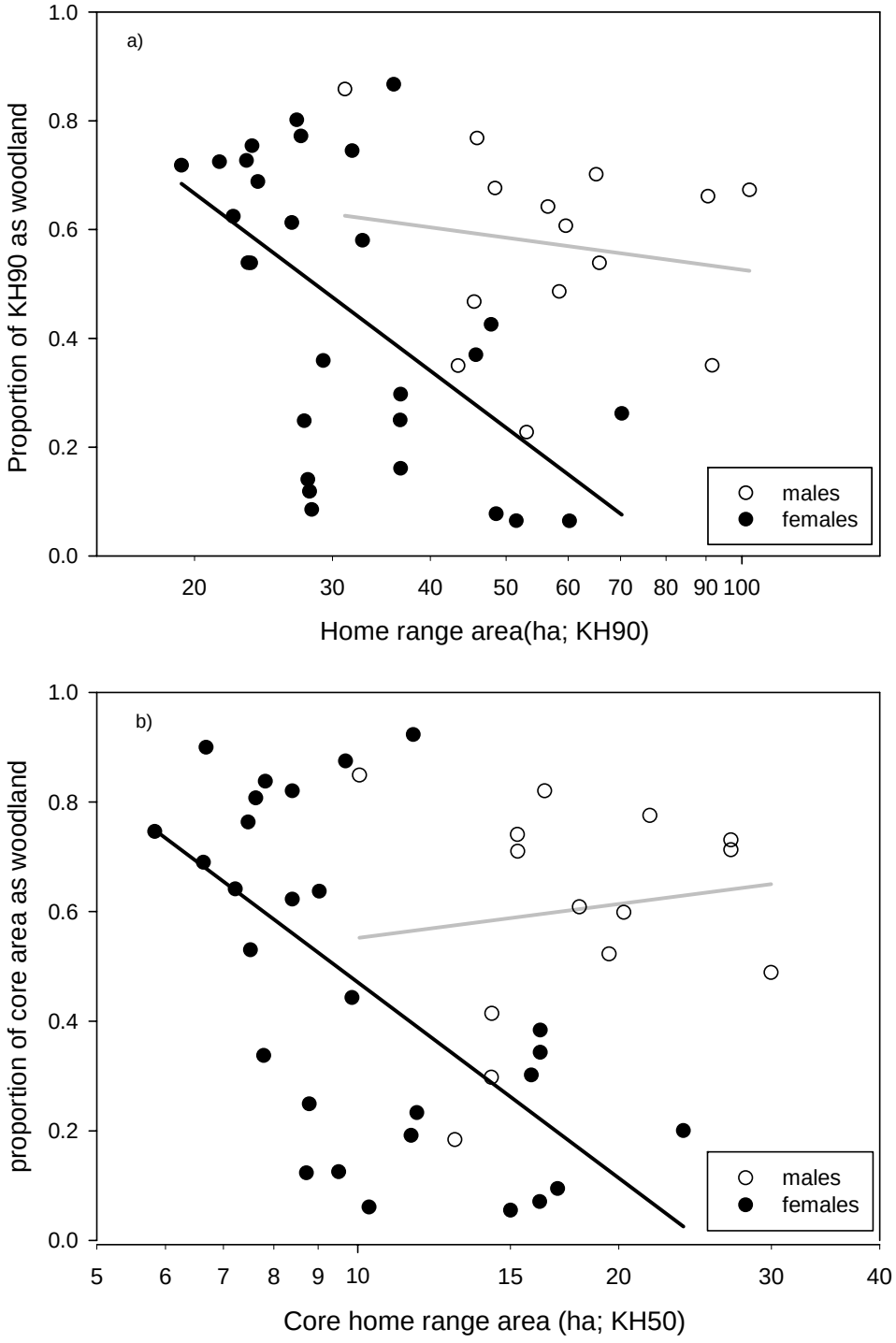

Figure 2. The relationship between the size of a) KH90 and b) core home ranges and the proportion of home range that comprises woodland vegetation for male and female echidnas *(Tachyglossus aculeatus)*. Values of home ranges and core areas are log-transformed to meet assumptions of normality.

Home range size and edges

There was a significant difference between males and females in the slopes of the regressions of edge area within the home range (KH90) against log KH90 (ANCOVA: $F = 19.64$; d.f. = 1,36; $P < 0.0001$, Fig 3a), and for the regressions of edge area within the core area (KH50) against log KH50 (ANCOVA: $F = 26.42$; d.f. = 1,35; $P < 0.0001$, Fig 3b). For males there was a strong positive correlation between the area of edge habitat within the home range and core area, and the size of home range and core areas. For females there was no correlation between the area of edge within the home range and core area and home range and core area size. The slope and intercepts of these regressions are summarised in Table 2. The mean area of edge in the core area (KH50) of female echidnas was 2.03 ± 1.08 ha, and in the 90% kernel (KH90) 5.91 ± 72.71ha.

When the area of edge in the home range and core areas was expressed as a proportion of the total area, the slope of the relationship between the proportion of edge and KH90 was non-significant for males (Fig 4a). For the core area (KH50) there was a significant positive relationship between the proportion of edge in KH50 and KH50 (Fig 4b). For females, these relationships were significant and negative (Fig 4a and 4b). The slope and intercepts of these regressions are summarised in Table 2.

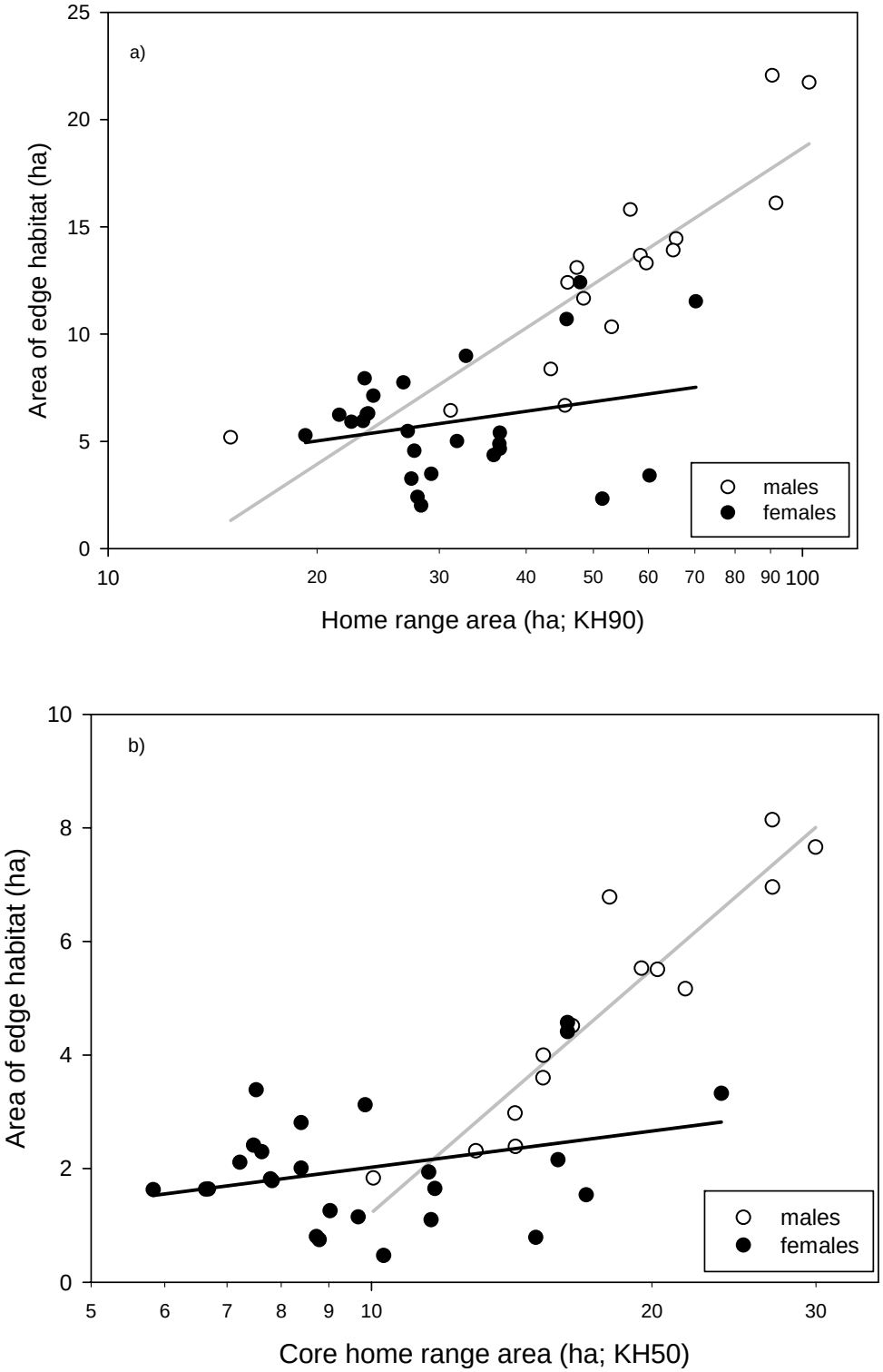

Figure 3. The relationship between the area of edge habitat and a) KH90 and b) core home ranges of male and female echidnas *(Tachyglossus aculeatus)*. Values of home ranges and core areas are log-transformed to meet assumptions of normality.

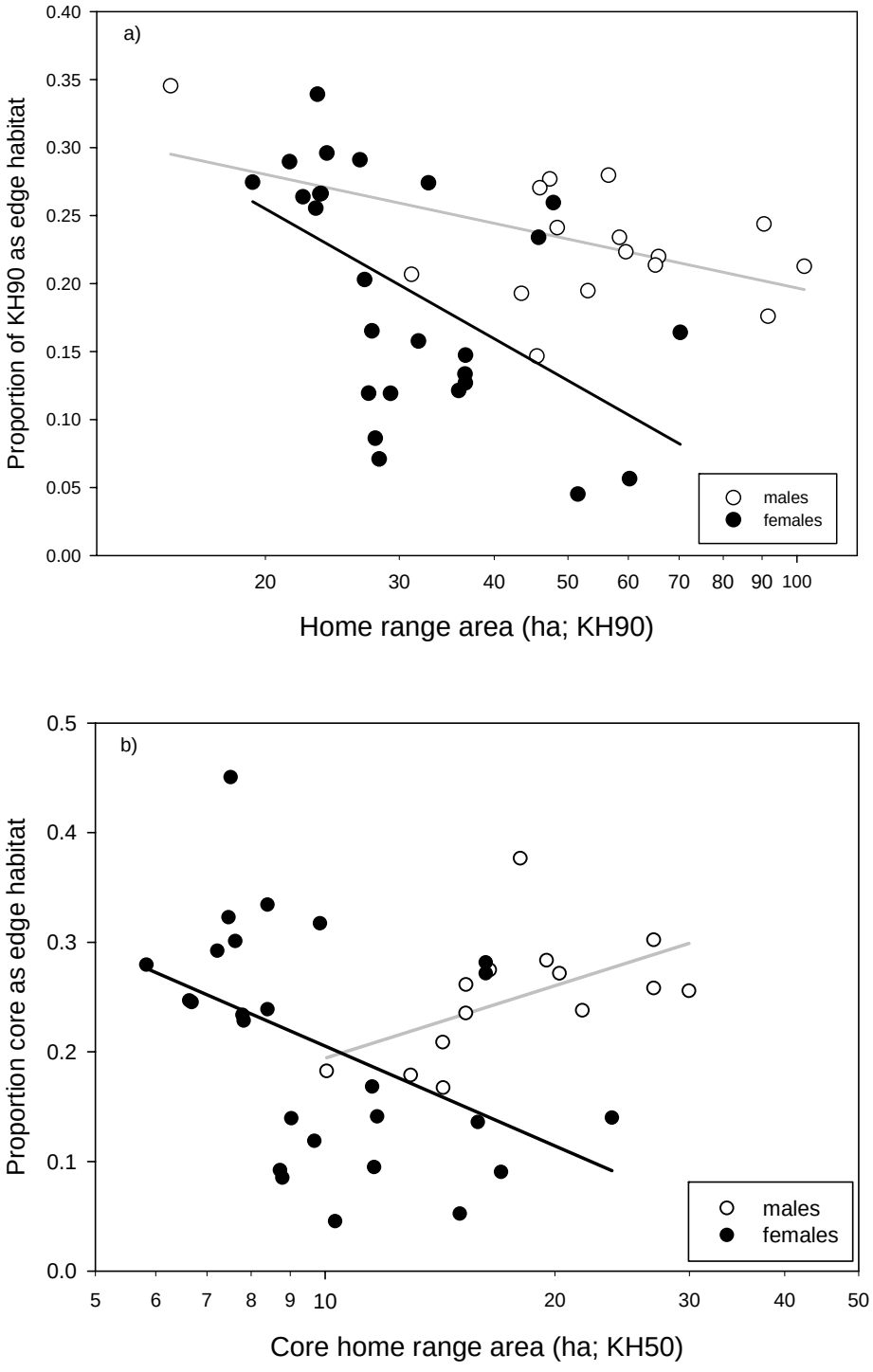

Figure 4 The relationship between the relative proportion of home range that is edge habitat and a) KH90 and b) core home ranges of male and female echidnas *(Tachyglossus aculeatus)*. Values of home ranges and core areas are log-transformed to meet assumptions of normality.

Table 2: Results of the analysis of the relationship between size of home range (KH90) and core home range (KH50) of the short-beaked echidna (*Tachyglossus aculeatus*), and total area of edge habitat and proportion of the total area edge habitat of KH90 and KH50.

Total edge area	Slope	Intercept	r^2	*P*	*Figure No.*
males					
KH90	+ 30.3	-161.2	0.82	< 0.0001	1a
KH50	+ 14.22	-69.90	0.87	< 0.0001	1b
females					
KH90	– 3.73	14.63	0.04	0.317	1a
KH50	+ 2.12	8.57	0.09	0.138	1b
Proportion of home range or core consisting of edge habitat					
males					
KH90	+ 0.001	-0.17	0.001	0.914	2a
KH50	+ 14.22	-69.90	0.29	0.048	2b
females					
KH90	– 3.73	14.63	0.29	<0.005	2a
KH50	+ 2.12	8.57	0.20	< 0.05	2b

Discussion

Home ranges calculated in this study were smaller than found in a previous study in the same area (Nicol *et al.* 2011). The smaller home ranges are a result of under-smoothing the data to emphasise areas of highest use (Worton 1989). Despite these differences, the results confirm the previous conclusion that male echidnas have significantly larger home ranges than females. In addition, the current analysis shows significantly different relationships between home range size and habitat for males and females. Larger home ranges incorporated more woodland for males but not females (Fig 1). The proportion of woodland is relatively consistent with different home range size for males, but for females, larger home ranges have proportionally less woodland (Fig 2). I interpret this as indicating that woodland represents better quality habitat than pasture and other vegetation, and that females have the smallest home range that can satisfy their resource requirements. Thus female echidnas whose home ranges are predominantly in woodland can obtain their resource requirements from a relatively small area, while those

whose home ranges are in pasture require much larger home ranges. For female echidnas, the relationship between home range size and vegetation type was stronger than that for home range size and edge habitat (Table 1), showing that female home range size is more closely related to vegetation than edge.

Males show a different pattern from females. Larger home ranges incorporate more woodland, but the proportion of woodland habitat in home range does not change. This appears to be a simple function of the scale of the landscape. As males and females have similar metabolic rates (Nicol and Andersen 2007a) and no difference in diet (Chapter 3) it appears that males have home ranges that are larger than is required to meet their energy requirements. This has also been observed in many other species including brown bears (*Ursus arctos*) (Dahle and Swenson 2003a) and raccoons (*Procyon lotor)* (Gehrt and Fritzell 1997), where the home ranges of males are not scaled to metabolic requirements or resource availability, but are a reflection of their promiscuous mating system in which males roam widely to gain access to females (Clutton-Brock 1989; Emlen and Oring 1977; Sandell 1989).

Echidnas display a high degree of home range fidelity from year to year and probably occupy the same home range for their entire adult life (Abensperg-Traun 1991; Augee *et al.* 1992; Nicol *et al.* 2011). During periodic arousals throughout hibernation echidnas may relocate to different hibernacula, but there is no seasonal change in the size of home ranges of hibernating and non-hibernating echidnas (Nicol *et al.* 2011), and the home range of echidnas must provide all resource requirements throughout the year including hibernacula, shelter sites and food. The distribution and availability of these resources will determine the quality of the habitat within a home range.

At the Tasmanian study site the predominant food items of echidnas are ants and the underground larvae of scarab beetles (Chapter 3). The distribution of ant nests and scarab larvae is likely to be dependant on vegetation. A study of the ant communities at the study site using pitfall traps indicated there was very little difference between woodland and pasture habitats (Yu Ka Ying 2010). Two prey species which contribute up to 50% of ant prey in the diet of echidnas (Chapter 3) were almost twice as common in woodland than pasture, and fewer ants were collected in pasture than woodland overall. Pasture habitat was more seasonal than woodland with more variation in ant species abundance in autumn and winter in pasture (Yu Ka Ying 2010). However, pitfall traps,

are not a good indicator of diet availability for echidnas, as they do not give an indication of the size, abundance and location of nests, providing information only on surface activity of individual ants (Ayre 1962; Greenslade 1964). Echidnas generally forage on ants within the nest rather than ants on the surface of the soil. Although a large proportion of the diet of Tasmanian echidnas consists of ants, the larvae of the pasture cockchafer beetle are also consumed in high numbers when they are available. The availability of these prey items varies seasonally (McQuillan *et al.* 2007), and they are more abundant in pasture and at the interface between pasture and woodland (P. McQuillan, pers.com.) (see below). Thus, a firm conclusion cannot be drawn about food availability, although echidnas foraging in woodland may have better access to several of their important prey species than echidnas foraging in pasture, and prey availability in woodland may be more predictable throughout the year as it is less influenced by seasonal variation than pasture.

Seasonality, albeit on a much larger scale than here, has been shown to influence the home range size of a number of species including brown bears (*Ursus arctos*) (McLoughlin *et al.* 2000), polar bears (*Ursus maritimus*) (Ferguson *et al.* 1999) and false antechinus (*Pseudantechinus macdonnellensis*) (Pavey *et al.* 2003). In seasonal environments, productivity is reduced during some periods of the year and, correspondingly, home range size increases as animals must range further to access sufficient resources (McLoughlin *et al.* 2000). Because there is more seasonal variation in ant abundance in pasture, echidnas with larger proportions of pasture in their home range may need larger home ranges to secure sufficient food.

Other aspects of woodland beyond food availability may be important. The high degree of structural complexity in woodland not only supports more prey items but also offers a range of sheltering opportunities for echidnas. Echidnas use shelters extensively for protection from predators and reducing the energetic costs of thermoregulation (pers. obs.; Beard and Grigg 2000; Beard *et al.* 1992; Bennett 1881; Grigg *et al.* 1992; Nicol and Andersen 2007a; Semon 1899; Smith *et al.* 1989; Wilkinson *et al.* 1998). Echidnas frequently change location during periodic arousals during hibernation, preferring cool sites early in hibernation, when ambient temperatures are still relatively high and moving to warmer hibernacula during the cooler months to maintain their body temperature within the preferred range of 6 - 10° (Nicol and Andersen 2007b). Access to a range of shelter sites is therefore critical for the echidna, which relies on hollow logs, depressions

under roots of fallen trees for shelter sites, including hibernacula (Abensperg-Traun 1991; Wilkinson *et al.* 1998). At the current study site echidnas shelter in large piles of dead trees and large rocks moved during land clearance, as well as *Lomandra* tussocks. Most of these features are more frequently located in woodland than pasture, although log piles and *Lomandra* patches are also present in pasture.

Although edge habitat has been shown to be an important resource for a large number of species there was no evidence to suggest that home range size of echidnas is determined by the amount of edge habitat within it. Edge habitats provide dense shelter for bridled nailtail wallabies *Onychogalea fraenata* (Fisher 2000), good browsing habitat for roe deer *Capreolus capreolus* (Saïd and Servanty 2005), more successful hunting opportunities for pumas *Puma concolor* (Laundré and Loxterman 2007) and a higher availability of dens for striped skinks *Mephitis mephitis* (Bixler and Gittleman 2000). For all of these species, smaller home ranges tended to have high proportions of edge, and larger home ranges had less edge available, a relationship similar to that found in female echidna home ranges. Edge habitat potentially offer a higher abundance of cockchafer larvae (P. McQuillan, pers. comm.), opportunities for foraging for pasture prey species with ready access to shelter positions within nearby woodland, and complex sheltering opportunities in the numerous large log piles present in the pasture/woodland interface. However for females the proportion of woodland (Table 1) explains more of the variation in KH50 and KH90 than the amount of edge does (Table 2). This suggests that proportion of woodland habitat has a greater influence on home range size than edge habitat.

Differential utilisation of resources through habitat partitioning may occur between conspecific individuals of the opposite sex. In the deer mouse *Peromyscus maniculatus* (Bowers and Smith 1979) and mountain pygmy-possum *Burramys parvus* (Mansergh and Scotts 1986), females may occupy small home ranges in good quality habitats while males are confined to the more marginal habitats, except during mating seasons. However, there was no evidence that habitat requirements of echidnas differ between males and females. The clear difference between male and female home ranges in relation to woodland habitat, as seen in figs 1 and 2, is consistent with the hypothesis that female home ranges are based on access to resources, but males are not. Both male and female echidnas must regain the fat stores lost during hibernation and reproduction in preparation for entering hibernation the following autumn but females have smaller home

ranges than males. Although females have high energetic costs during late lactation (Nicol and Andersen 2007a), males have higher energy expenditure during the mating season; it seems that males simply have larger home ranges than are required to meet their food and shelter requirements.

Like other k-selected 'slow' (Heppell *et al.* 2000) species, female echidnas appear to adopt an area-minimising approach to accessing resources, so that their home range contains all resources for survival and reproduction in as small an area as possible (Mitchell and Powell 2007). In order for successful reproduction and subsequent hibernation, female echidnas may be reliant on accessing areas of high productivity and shelter availability within a relatively short distance from the nursery burrow in order to reduce the energetic costs in the trade-off between suckling young and foraging (Clutton-Brock *et al.* 1982). These habitats reduce foraging effort during reproductive years as well as providing sufficient resources for young after weaning (Rismiller and McKelvey 2009). The reproductive success of male echidnas is based on maximising access to females and is reflected in their large home range and lack of relationship with proportion of woodland habitat. This pattern is common in solitary species, where the spatial distribution of females is determined primarily by the distribution of resources and the distribution of males is based on maximising access to females (Clutton-Brock 1989; Emlen and Oring 1977; Sandell 1989). The promiscuous mating system of solitary, sparsely distributed species like echidnas results in their spatial organisation ultimately being driven by females, a pattern observed in a number of other species e.g. racoons *Procyon lotor* (Gehrt and Fritzell 1998), fossa *Cryptoprocta ferox* (Hawkins and Racey 2009), bobcats *Lynx rufus* (Ferguson *et al.* 2009) and Eurasian red squirrels *Sciurus vulgaris* (Verbeylen *et al.* 2009).

Conclusion

The home range size of male and female echidnas show significant differences in both size and their relationship to the habitat located within them, and this highlights the need to examine the sexes separately when considering the spatial ecology of a species. Sex linked differences in the relationship between habitat quality and home range size has been demonstrated in a number of other species, and it would be expected that it would occur in most other solitary species with a promiscuous mating system. This is the

first time it has been demonstrated in a myrmecophagous species. The factors that drive the spatial distribution of female echidnas may differ from that observed in other species however. Strong relationships between habitat quality and home range size in other species are commonly linked with food availability (e.g. Herfindal *et al.* 2005; Broughton and Dickman 1991; McLoughlin *et al.* 2000; Tufto *et al.* 1996). However, the size of female echidna home ranges may be driven more by the availability of sheltering opportunities than food resources. The prey of echidnas is widely distributed and generally abundant. There is no difference in the size of home range of echidnas between hibernation and the active period (Nicol *et al.* 2011), and echidnas frequently re-use the same shelter (Abensperg-Traun 1991; Wilkinson *et al.* 1998; pers. obs.) indicating that shelters may be a limiting resource, and it seems plausible that home range size may be determined primarily by shelter availability and to a lesser degree, food availability. This is in agreement with Smith *et al.* (1989) who suggest that habitat is selected by echidnas primarily on the basis of shelter availability and secondarily by the availability of food.

Whilst it is not possible to identify absolutely what are the limiting resources that determine the size of an echidna's home range through kernel analysis alone, it is clear that a number of potentially limiting resources are associated with woodland habitat. There are significant differences between males and females in the relationship between the amount of woodland and the size of home ranges, with an inverse relationship between female home range size and proportion of woodland, indicating that woodland is higher quality habitat than pasture.

Appendix to Chapter 4

Trial of use of GPS loggers to determine activity of short-beaked echidnas (Tachyglossus aculeatus)

Appendix to Chapter 4

Trial of use of GPS loggers to determine activity of short-beaked echidnas (Tachyglossus aculeatus)

As the data used for estimation of home ranges of short-beaked echidnas in this study were obtained by radio tracking animals during the daytime, they may not be a true reflection of habitat use, and may be biased towards use of shelters. In order to obtain a better idea of how animals used their habitat a number of echidnas were equipped with GPS loggers to determine use of different vegetation types within their home range. GPS loggers have not previously been deployed on echidnas, so this was in part a preliminary trial of the practicability of using GPS loggers on echidnas.

GPS loggers

Two different types of μGPS loggers were deployed on echidnas. The first, a unit designed for use with flying birds (Steiner *et al.* 2000), was supplied by NewBehavior (http://www.newbehavior.com/products/discontinued-products/micro-gps) and weighed 39 g including the battery. The second was designed specifically for use on echidnas in this project by Sirtrack Ltd, Havelock North, New Zealand. The total weight of the unit was 105 grams. For both logger types, loggers were screwed to a small aluminium plate glued to the upper back of the echidna, allowing easy exchange of loggers in the field.

GPS logger deployment

Details of the loggers and their deployment are summarised in Table 3. The mean logging period obtained with the NewBehavior loggers was only 9.1 h (maximum 17.2h). The SirTrack loggers performed better, but the average logging period was still only 32.7h, which was not long enough to undertake a meaningful analysis of habitat use, particularly a comparison of daytime and night time points.

Both types of logger failed when echidnas were in shelters and no GPS signal could be received. Under these conditions the software locked up, and the batteries quickly drained, regardless of the fix interval. Thus the only long records may have been unrepresentative of the range of normal echidna activity patterns. Despite the considerable time and effort spent in deploying the loggers there were insufficient data to

undertake any of the planned analyses, such as comparisons between individuals, seasons, or sexes, while location accuracy of SirTrack loggers (± 17.3 m under optimal conditions) was too poor to provide as useable indication of activity. This trial showed that it was possible to attach GPS loggers to echidnas. The units remained attached to animals for 3+ days and their retrieval was relatively easy.

Table 3. Summary of number of times deployed, error, length of time logged and % of successful fixes of the two GPS loggers deployed on echidnas *(Tachyglossus aculeatus)*.

Logger type	Fix interval	Mean error*	Total deployments	No. of individuals	Average time logged (hours)	successful fixes	No. >48 hrs
NewBehavior	10 sec	4.1 m	23	3	9.1 (0.9 – 17.2)	46%	0
SirTrack	2 mins	17.3 m	14	7	32.7 (11.1 – 59.7)	80%	4

*Good reception conditions

Limitations of habitat use from fixed data points

Incorporating GPS technology into home range analysis should have helped to address the question of what determines home range size, and reveal if edge habitat is used preferentially over woodland. However, limitations with the function of GPS loggers prevented analysis of home range use. By identifying different habitat types within each home range, in Chapter 4, I was able to partly reduce the bias that can be introduced when attempting to determine the spatial distribution of animals within their environment. Home ranges estimated from radio telemetry data are largely continuous in space, and in their most basic form are the sum of all places an animal goes (Powell 2000). As such, they assume a uniform use of space within home range boundaries, and thus include patches of resource rich habitat embedded in less valuable area which are primarily used for travel, or not at all (Mitchell and Powell 2008). By selecting a less conservative smoothing parameter (h_{ref} *50)* and a smaller resolution grid (g20) I was able to construct home ranges that identified areas of more intense use with increased precision (Seaman and Powell 1996). By then identifying different habitats within home ranges, I was able to discern with greater accuracy, the location of potential resource bearing, high quality patches that shape the home ranges of female echidnas. However, the data used to construct home ranges showed it was highly skewed towards sheltering behaviour. Of the 2182 locations recorded, only 380, or 21%, were from active animals

and the remaining 1802 locations were from animals observed whilst sheltering. Thus echidnas may actually have different diurnal and nocturnal home ranges. Radio tracking at night was not possible, so I hoped that by equipping echidnas with GPS loggers, I could obtain information on nocturnal movements and home range use, in addition to determining finer scale utilisation of these high quality habitats. The frequent sampling rate of GPS loggers is likely to capture areas of important resources which may be used only occasionally (Kochanny *et al.* 2009) or during periods when traditional methods of location are not being deployed (i.e. night time). However the units failed to collect sufficient data over extended periods to be able to carry out any analysis.

The relatively small distance travelled by echidnas was problematic when analysing data from the GPS loggers. Separating travelling, foraging and sheltering behaviours on the basis of distance travelled was often not possible due to the relatively slow movements of animals, combined with the positional error of the GPS logger itself, and short battery life. Echidnas typically forage by moving slowly across the ground, frequently poking their beak into the soil in search of prey items and then stopping to dig down into the soil to extract ants or larvae, and they may spend many minutes foraging and feeding within an area of approximately 10m x 10 m (pers. obs.). The GPS tracks of foraging animals are often indistinguishable from those of sheltering echidnas. Sheltering beneath logs or rocks generate fixes with a high positional error, with correspondingly high inter-fix distances, and rapidly depleted the battery. This made movement analysis impossible as foraging and sheltering may be indistinguishable. Improved technology has reduced battery consumption and units may continue to collect data after an animal shelters for a prolonged period. This means that a logging event is not terminated during sheltering and it is likely to be feasible to use GPS loggers to identify sheltering, foraging and travelling behaviours of echidnas.

Although the particular units used did not collect sufficient data for analysis, the GPS loggers did remain attached to echidnas for several days. This indicates that there is scope for further application of GPS technology in future research into the spatial ecology of echidnas, particularly as technology continues to deliver smaller units with significant improvements in battery life.

Chapter 5a

Latrine use by the short-beaked echidna *Tachyglossus aculeatus*

Sprent JA, Andersen NA and Nicol SC, 2006. Latrine use by the short-beaked echidna, *Tachyglossus aculeatus*. Australian Mammalogy **28**, 131-133.

Published as a note to Australian Mammalogy

LATRINE USE BY THE SHORT-BEAKED ECHIDNA, *TACHYGLOSSUS ACULEATUS*.

Sprent JA, Andersen NA and Nicol SC, 2006. Latrine use by the short-beaked echidna, *Tachyglossus aculeatus*. Australian Mammalogy **28**, 131-133.

Key words: echidna, *Tachyglossus aculeatus*, latrine, home-range, behaviour, social structure

In mammals the basic functions of defaecation and urination have an inherent secondary function of chemical communication (Eisenberg 1981), and mammals have evolved a variety of behaviours based on these means of communication. Many mammals, particularly carnivores such as European badgers (*Meles meles*) (Stewart et al. 2002), honey badgers (*Mellivora capensis)* (Begg et al. 2003), and quolls (Kruuk and Jarman 1995; Oakwood 2002), but also non-carnivorous species such as rabbits (Sneddon 1991), leave accumulations of faeces, or latrines, that may serve a number of social functions, such as the marking of territories and the maintenance of dominance hierarchies.

In the course of radio-tracking short-beaked echidnas (*Tachyglossus aculeatus setosus*) at our study site on a grazing property in the southern Tasmanian midlands we have discovered what are clearly echidna latrines. The approximately 1000 ha study area comprises a mixture of pasture and areas of remnant dry sclerophyll forest and woodland dissected by numerous gullies with sandstone outcrops, and contains about 100 resident echidnas. The majority of latrines were found under windrows piled up during land clearing activities, with one found under a single log lying amongst thick bracken. In a brief survey of part of the study area with cliffs and many rocky outcrops we found a latrine under a sandstone overhang. The presence of a latrine is indicated by an area of

approximately 25 cm diameter which is clear of vegetation and sticks. Some sites have several (2-3) distinct latrines close together – less than 1 m apart – on the opposite sides or opposite ends of a log, but within the same log pile. Scats may be visible on the surface or buried several centimetres into the soil, which is soft and full of exoskeletons. Echidna scats consist mostly of soil, making them quite difficult to see; they can be neat cylinders up to around 5 cm long or simply resemble a pile of hardened soil. Upon close examination exoskeletons of ants and other chitinous prey items can be clearly seen. The soil-exoskeleton mix extends down around 5-10 cm and may contain crusty layers, probably caused by moisture – rain or urine –breaking down scats and the slurry then hardening as it dries. The latrines can have a strong 'echidna' odour and may be damp with rainwater or urine.

Of the 22 latrines discovered, four were found while tracking a single individual male (1E7C), as he was sheltering in the latrine or very close to it when located. Four more were located within his known home range. One latrine is within an area where we regularly find five animals, including 1E7C and lies very close to the home ranges of another ten echidnas. Two other animals have been found within the area, one of which is known to have a home range 2 km distant.

In order to relate the amount of faeces to the number of defaecations, and thus the number of visits, we used the weight of what we judged to be a reasonably representative dry scat found next to a latrine as a standard scat. The ends of this scat were tapered and it appeared to be complete and weighed 46 g. One latrine was cleared eight times between May 2002 and January 2005 yielding more than 1 kg of faeces in total which would represent a minimum of 20 repeated visits. Two sites, one of which had three distinct latrines within an area of 2 m^2, and another with two latrines within an area of 1m^2, both yielded 2 kg, representing more than 40 visits. Given the size of these deposits it is surprising that the only previous indications of the existence of echidna latrines are a brief comment by Griffiths (1968) that caves in Western Australia "contained pounds of echidna scats" and collections of scat samples from echidnas in the Northern Territory that contained up to 35 pieces (Griffiths et al. 1990).

Our first observation of a latrine was in 2002 and this latrine was still in use in 2005. We checked seven of the latrines approximately every 30 days from September 2004 to

January 2006, and found that they were continuously used between August and February. Between February and July Tasmanian echidnas hibernate (Nicol and Andersen 2002) and the latrines were not used during this period.

It is reasonable to assume that echidna latrines, like those of other mammals, have a social function. Burying faeces prolongs the period during which the odour remains strong (Eisenberg 1981), while their location gives them protection from weathering, particularly from rain, which rapidly breaks down echidna scats (Rismiller 1999; Smith et al. 1989). Scats are also deposited individually, with no association with latrines, and thus a key to understanding the role of latrines will be information on the circumstances in which echidnas use latrines, compared with when they do not.

Echidnas are considered to be solitary except during the breeding season (Augee et al. 1975; Griffiths 1978), although we have occasionally observed animals in close proximity to each other (<1 m) outside the breeding season (Andersen, Sprent and Nicol, unpubl. data). Echidnas have well defined home ranges that overlap significantly (Abensperg-Traun 1991; Augee et al. 1992; Augee et al. 1975; Nicol and Andersen, unpubl. data; Wilkinson et al. 1998), but there has been no previous evidence of any social structure in echidnas in the wild. Male dominance hierarchies may be important during echidna reproduction; during mating males may jostle each other for access to females and this behaviour has been observed in the field (Rismiller and McKelvey 2000), and in captivity (Boisvert and Grisham 1988; Brattsrom 1973).

Even solitary animals will have a social structure to maintain spacing and maximise resource utilization (Eisenberg 1981), and require an effective communication system to maintain their social organization and ensure reproductive success. Chemical or olfactory communication enables solitary animals to leave messages that are relatively long-lasting, can be 'read' later, and can also be used at night, underground or in dense vegetation (Begg et al. 2003). Olfactory communication clearly plays an important role in echidna mating behaviour, with males being attracted to females by scent (Rismiller 1992).

Begg et al. (2003) list a number of possible functions of scent marking in honey badgers which could also apply to echidna latrines: indicating the presence of dominant males, signalling of reproductive status by females, signalling home ranges and thus

allowing spatio-temporal separation of neighbours, or signalling where an individual is feeding, thereby enabling individuals to partition resource use and increase foraging efficiency. These different hypothesised functions lead to specific predictions about patterns of use (Begg et al. 2003) which can be tested by field observations and knowledge of which animals are using the latrines. For example, a role in signalling reproductive status by females seems unlikely as latrine use is not confined to the mating period (early June to mid-September, Nicol and Andersen in press) but occurs throughout the active period. Furthermore, in Tasmanian echidnas the female normally mates within one or two days of arousal from hibernation, (Nicol et al. 2005), sometimes before she has moved from her hibernaculum. In order to elucidate the role of these latrines, and gain insights into echidna social structure, we shall be continuing field studies, which will include the use of DNA analysis to indicate which animals are using latrines.

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Chapter 5b

Spatial distribution and possible role of latrines in the spatial ecology of echidnas

Abstract

Large accumulations of scats of the short beaked echidna (*Tachyglossus aculeatus*) have been noted at several sites within Tasmania. To investigate possible roles of latrines in the spatial ecology of echidnas, the location of latrines in relation to vegetation type, as well as the location of core home ranges of male and female echidnas, was mapped at a site located in the Southern Midlands of Tasmania. The density of latrines was not the same in all patch types. Latrines were approximately four times more densely distributed in scrub patches than in patches of open habitat and thick bush, suggesting that scrub patches are more favoured locations for latrines than patches of open pasture or thick bush. Twenty adult individuals - nine females and 11 males, had their core home range overlap with the location of latrines. Latrines occurred most frequently where two core areas overlapped, and were much less likely to be found in areas where four core areas overlapped. This may indicate that there is no benefit of maintain latrines sites if no other animals live nearby, and in areas of high numbers of animals, latrine use and territorial behaviour may be abandoned due to the high energetic costs of over marking scats from other individuals. The higher densities of latrines in scrub woodland, and the higher frequency of latrines in areas of intermediate rather than high echidna density, suggest that through signalling presence in high quality areas, female echidnas may exclude other individuals. In turn, male echidnas could use information from latrines to maintain their home ranges to maximise access to females.

Introduction

Many mammal species use accumulation of faeces in localised defecation sites known as latrines, as a method of chemical communication. Such olfactory signals often play a key role in intraspecific communication, and are used by a broad range of mammalian taxa including honey badgers *Mellivora capensis* (Begg *et al.* 2003), European badgers *Meles meles* (Stewart *et al.* 2002), rabbits *Oryctolagus cuniculus* (Sneddon 1991), quolls *Dasyurus sp.* (Claridge *et al.* 2004; Kruuk and Jarman 1995; Oakwood 2002), and otters *Lutra canadensis* (Rostain *et al.* 2004). Olfactory communication enables solitary animals to relay messages to conspecifics in the absence of the signaller. The persistence in the environment of these chemical cues permits long-term broadcast of information (Fornasieri and Roeder 1992; Salamon and Davies 1998). Olfactory signals may be present in urine and/or faeces or produced from specialised scent glands (Bekoff 2001; Putman 1984; Zabaras *et al.* 2005). These signals are deposited in the environment through waste elimination or rubbing exudates from scent glands onto objects; exudates from scent glands located around the anus may also be deposited on scats when they are eliminated.

The location of latrines within an animal's landscape can give an indication of their function. Concentrations of latrines along territorial borders where they are more likely to be encountered by conspecifics suggest a role in territorial defence (Brashares and Arcese 1999), and increased density of latrines in core areas of home ranges may function to demarcate areas of exclusive use (Jordan *et al.* 2007; Wronski and Plath 2010). However, latrines and other scent marking behaviours may have a limited role in territorial marking, instead functioning as a site of information exchange. They may signal reproductive status (Darden *et al.* 2008), dominance (Sneddon 1991), or enabling the partition of resource use by signalling where an individual is feeding or quality of food (Begg *et al.* 2003; Henry 1977; Kruuk 1995). Latrines provide a medium for exchange of information between individuals negating the need for direct contact. Transmission of information regarding social hierarchy at latrines (Kruuk 1972; Rostain *et al.* 2004) may reduce the chance of physical altercations, or minimise harassment of female by males attempting to determine their reproductive status (Wronski *et al.* 2006).

The short-beaked echidna (*Tachyglossus aculeatus*) is a solitary species in which individuals occupy well defined home ranges. Echidnas shows high home range fidelity and home ranges are stable over many years (Nicol *et al.* 2011). The home ranges of females appear to be based on access to resources, while the home ranges of males appear to be based on access to females (Chapter 4), a pattern that is particularly common in solitary carnivores (Clutton-Brock 1989; Sandell 1989). How this spatial organisation is maintained by echidnas however is not clear. Aggressive or agonistic interactions have not been observed, and the distribution of the species is not restricted by habitat: echidnas are almost ubiquitous in their distribution across Australia (Augee 2008; Griffiths 1968).

The echidna cloaca is surrounded by scent glands that produce a secretion rich in organic compounds, particularly volatile carboxylic acids and other odorous compounds (Harris 2009). The function of these compounds has yet to be determined. It seems likely that the location of cloacal glands would result in the transfer of chemical secretions onto scats, either during defaecation or by marking scats after elimination. Cloaca wiping behaviours have been observed in captive and free ranging echidnas (Boisvert and Grisham 1988; Dobroruka 1960). Similarly, the faeces of domestic dogs *Canis familiaris* receive a smear of anal sac secretion as they are passed (Ashdown 1968), and European

badgers *Meles meles* deposit strong smelling secretions from their anal glands over the top of scats previously deposited, or on the ground nearby (Kruuk and Hewson 1978).

Large accumulations of echidna scats have been noted at several sites within Tasmania (Chapter 5a; Grove *et al.* 2006; Sprent *et al.* 2006). The discrete nature of their deposition, usually confined to within an area less than 0.5 m^2, indicates that deposition is not a random event, but occurs through repeated visits to the site by single individuals or multiple echidnas (Sprent *et al.* 2006). Latrines are used continuously between August and February (Sprent *et al.* 2006) which is when echidnas are active and not hibernating (Nicol and Andersen 2007a). Located under conspicuous features, usually large logs, latrines are likely to be areas echidnas may frequently visit since fallen logs offer plentiful opportunities for sheltering. The presence of these latrines and the presence of cloacal scent glands suggest that latrines may play a role in chemical communication between individuals, by acting as centres of information exchange through which they control and maintain their spatial organisation.

Echidnas frequently re-use the same shelter location and different individuals have been observed in the same locations at different times (pers. obs), indicating potential for information exchange at latrines. For solitary animals such as the echidna there may be limited chance of direct contact between individuals, and they may also be frequently out of range of visual or acoustic signals. Therefore a communication network based on olfactory signals that persist after the signaller has left may be particularly important. In systems where several individuals visit and use common latrines, latrines can form a network of communication between individuals and potentially the whole population. As latrines build up, so does information regarding animals' activities at the site, and a timeline of events develops (Darden *et al.* 2008).

This study builds upon a previous description of echidna latrines (Sprent *et al.* 2006). It examined the spatial distribution of latrines and some of the possible roles of latrines in the spatial ecology of echidnas in a woodland-pasture grazing system in southern Tasmania. I investigated distribution in relation to vegetation type, as well as location of core home ranges of echidnas, to suggest possible roles of latrines in the spatial ecology of echidnas. Scent marking may involve costs in terms of both the production of marking substances and the time taken to deposit marks, and consequently animals may be forced to make strategic decisions about placing marks. Assuming that

the value or quality of resources varies between different vegetation types, the distribution of latrines will vary with vegetation type if echidnas are using latrines to signal resources use. Latrines would be expected to be more densely distributed in good quality habitats. If latrines are used by echidnas in the defence of home ranges, advertising the continued presence of home range owners, latrines should be more frequently located in areas of overlapping home ranges.

Materials and Methods

Fieldwork was carried out at Lovely Banks, a grazing property in the southern midlands, 55 km north of Hobart, Tasmania (longitude 147 14', latitude 42 25'S). Vegetation at the site consists of remnant dry sclerophyll woodland dominated by *Eucalyptus amygdalina* interspersed with improved and native pasture and patches of *Acacia dealbata*, *Casuarina verticilata* and *Lomandra longifolia*. The site is intersected with numerous gullies, caves and sandstone outcrops and ranges in altitude from 200 to 400 m asl.

Latrine survey

Latrines were mapped in October 2010 by traversing on foot a section of the field site, approximately 62 hectares in size, known from previous studies to contain the home ranges of numerous adult male and female echidnas. Because the area had been subject to intensive study it could be confidently assumed that the core areas of animals' home ranges were located within this area: there were few individuals for which there was not a reasonable estimate of their home range. The survey area was located in an innermost region of the study area to reduce the chances that the area would be used by echidnas from nearby less intensively studied areas.

Previous observations of latrines, initially discovered in the course of radio tracking echidnas, revealed that they were exclusively located under fallen logs and trees, and large slabs of rock (Chapter 5a; Sprent *et al.* 2006). These objects have a space beneath them large enough to allow an echidna to enter, and have a loose soil base. Latrines typically have scats on the soil surface, but may also be buried (Grove *et al.* 2006; Sprent *et al.* 2006). To locate echidna latrines, the survey area was systematically searched for logs and large rocks. The woodland habitat had a relatively open understorey and it was possible to visually scan the area for fallen logs and large rocks, and then

check for latrines beneath them. Throughout areas of pasture, fallen logs and rocks and large windrows created during land clearance operations were also examined. The vegetation structure was open enough that few logs and rocks would have been inadvertently missed when checking for latrines. The presence of a latrine was verified through observation of a number of the characteristic cylindrical scats of echidnas (Triggs 2005) lying on the soil surface, and/or scats dug under the soil. If no scats were visible of the soil surface of an otherwise likely latrine, the top 5 cm of soil was probed by hand to check for buried scats. Once a latrine was located, the type of habitat features it was associated with was noted. The position was recorded using a hand held GPS and the coordinates later uploaded into a GIS package.

GIS and Mapping Core Areas

Using a high resolution QuickBird image in ArcGIS, the vegetation within the site was allocated to one of three categories based on the density of tree cover. The site consisted of patches of woodland separated by open pasture (see Fig. 1). To determine the density of trees across the survey area, 100 m transects were drawn across blocks of vegetation on the image, and the number of trees intersected by the transect counted. Areas with between 0 and 4 trees on a 100 m transect were classified as *open*, 5 to 12 trees classified as *scrub* and areas with 13 or more trees were classified as *thick*. Based on this assessment, blocks of differing tree density were digitised, and the total area of each category calculated. Latrine density in each category was then determined.

Echidna location data was collected between August 2004 and October 2010. These data were incorporated into a long term data base of all echidna locations recorded at the field site since 1996. Echidna location data for home range analysis was drawn from this database, which has been used in previous home range analyses (Nicol et al 2011, and Chapter 4). All echidnas observed in the study area could be positively identified through subcutaneous passive transponder tags (LifeChip, Destron Fearing, MN, USA). To determine how many core home ranges overlapped with latrine sites the core home ranges of individual echidnas were estimated. Location data was analysed using the Home Range Extension (Rodgers and Carr 1998) in ArcView GIS 3.3. As in a previous echidna study (Nicol *et al.* 2011), a fixed kernel method and the reference (h_{ref}) smoothing parameter was used. The 50% kernel area was estimated as this is generally

considered to be the core of an animal's home range, and is more likely to be used exclusively by an individual (Cavalcanti and Gese 2009; Cimino and Lovari 2003). Core home ranges were calculated only for those individuals for which there was 20 or more locations relatively evenly spread throughout the year. This number was deemed appropriate to provide unbiased estimates of echidna home range by (Nicol *et al.* 2011) who used an incremental analysis (Kernohan *et al.* 2001) based on the assumption that home range estimates reach an asymptote with adequate sample size (McLoughlin and Ferguson 2000).

Home ranges were constructed using all available location data collected from the study site. Echidnas have a high degree of home range fidelity over long periods (Nicol *et al.* 2011) and probably spend their entire adult life within the same home range (Abensperg-Traun 1991; Augee *et al.* 1992). However, to ensure that the estimates reflected the current population only data from individuals that had been observed during the previous 5 years were included. Latrines and core home ranges were plotted in ArcGIS 9.2. For each latrine, the tree density and the number of core home ranges which overlapped the latrine location was determined.

Statistical Analysis

A Chi-squared test was used to determine if latrines were randomly distributed across the three different vegetation patch classes (open, scrub, thick), the null hypothesis being that density is the same in all patches. A factorial analysis of variance (ANOVA) was used to test for the effect of differences between vegetation type and sex of home range owners against the number of core home ranges associated with each latrine. Analysis was performed using the software package Statistica 6.1 (Statsoft, Tulsa, Oklahoma).

Results

Sixty four latrines were found in the survey area, and all were located under large logs or fallen trees. Latrine distribution appeared to be related to physical features of the environment. There were significant differences between densities predicted from a random distribution and actual densities of latrines within the three patch types ($\chi^2_2 = 34.83$, $p < 0.005$). Latrines were approximately four times more densely distributed in

scrub patches than in patches of open habitat and thick bush (Table 1). This difference in the density of latrines in patches of different vegetation type is unlikely to be a reflection of the abundance of fallen logs. Whilst logs were abundant in scrub, where latrine density was greatest, they were also abundant in thick bush which had the lowest density of latrines. Although pasture areas contained some fallen logs, no latrines were found under them, indicating that presence of latrines was not a function of the scarcity of log shelters. The data suggests that logs within scrub patches are more favoured locations for latrines than logs within patches of open pasture or thick bush.

Table 1. Area of each patch type, numbers of echidna latrines within each patch type and density of echidna latrines.

Patch type	Area (ha)	Number of latrines	Latrine density/ha
Scrub	16.56	34	2.05
Thick	25.37	12	0.47
Open	31.94	18	0.56
Total	73.87	64	0.87

Over the five year period leading up to the study a total of 32 echidnas were observed within the survey area. Twenty of these animals - nine females (Fig 1) and 11 male adult echidnas (Fig 2), had their core home range overlap with the location of latrines. Two males and six females had been observed within the survey area although their core home ranges were located out of the survey area. A further four juvenile animals were observed within the area of latrines. These juveniles were the only individuals for which there were insufficient locations available to estimate home ranges. Thus, it was unlikely that there many other individuals that were unaccounted for within the area, and I could be confident that there were few, if any, other resident echidnas within the area. Only two of the 64 latrines did not fall within a known core area.

The number of core areas which included any specific latrine did not vary significantly with patch type ($F_{2,122} = 0.59$, $P = 0.55$), and there was no significant effect of the sex of the owners of home ranges on the number of core areas present at latrines ($F_{1,122} = 0.0006$, $P = 0.98$). There was also no interaction between patch type and sex of home range owner on the number of core areas overlapping a latrine location ($F_{2,122} = 2.32$, $P = 0.10$). The mean numbers of female and male core areas associated with latrines in different patch types are summarised in Table 2.

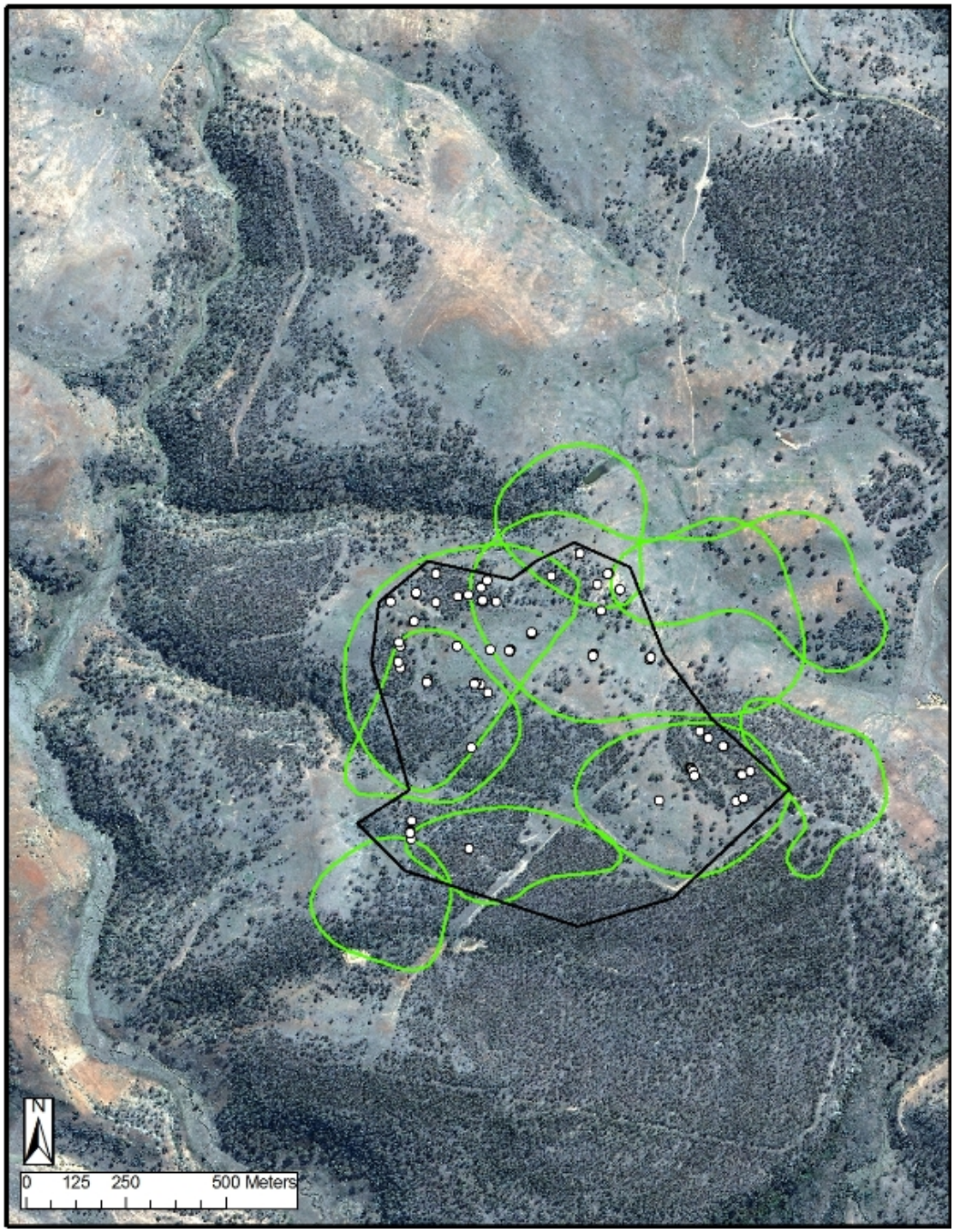

Figure 1. Location of latrines (white circles) and core home ranges (green lines) of known female echidnas (*Tachyglossus aculeatus*). The black line indicates the latrine survey area.

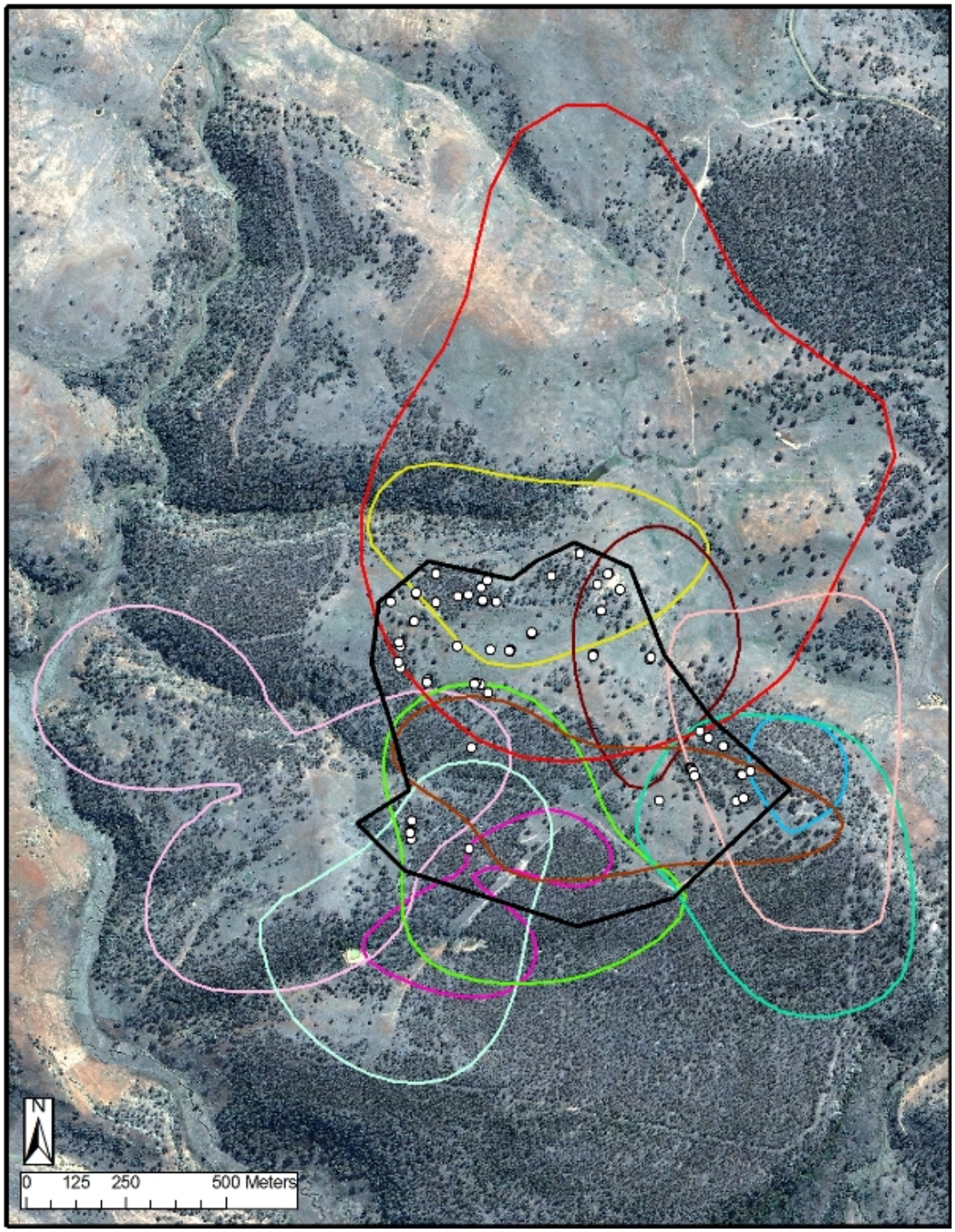

Figure 2. Location of latrines (white circles) and core home ranges of known male echidnas (*Tachyglossus aculeatus*). The core home ranges of individual echidnas are distinguished by different coloured lines. The black line delimits the latrine survey area.

Table 2. The mean number of echidna core home ranges that contained latrines located in patches of different vegetation type. N = number of latrines.

Patch type	N	Mean number of known core home ranges (females)	Mean number of core known home ranges (males)
Open	19	2.53 ± 0.18	2.11 ± 0.13
Scrub	33	2.27 ± 0.20	2.18 ± 0.12
Thick	12	1.83 ± 0.11	2.33 ±0.26

Fig. 3 shows the number of latrines that were overlapped by one, two, three of four core areas. Latrines occurred most frequently where two core areas overlapped, and were much less likely to be found in areas where four core areas overlapped. Whilst it is impossible to verify the presence of all animals living within the study area, I am confident that I have identified the majority of individuals with core home ranges overlapping latrines.

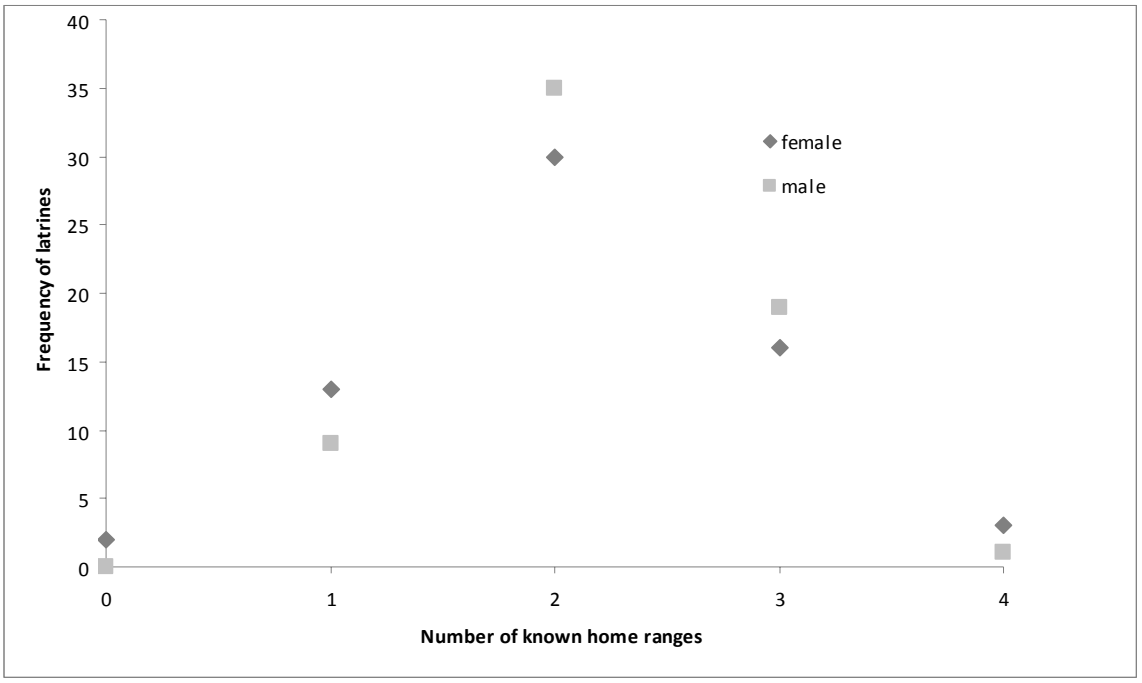

Figure 3. The frequency that echidna latrines were overlapped by 1, 2, 3, or 4 core home ranges of male and female echidnas.

Examination of all records from the study site showed that of the 5642 observations made over 15 years, echidnas were found in, or in close proximity to, latrines only 13 times: nine of these observations were of males, and only four were of females.

Discussion

Echidna latrines were not randomly distributed throughout the study area. All echidna latrines were located under large logs or fallen trees, which are important echidna shelter sites (Wilkinson *et al.* 1998). Many locations that appear to offer good protection from weather and disturbance for a sheltering echidna had no evidence of scats either within them or nearby, which indicates that latrines are unlikely to simply be the consequence of repeated use of a shelter and normal elimination behaviour. If this were the case, most shelter sites should have scats within them. Windrows and log piles generally have numerous separate sheltering locations within them. In these piles there is often a single latrine with no evidence of scats in other shelter locations within the same log pile. The large amounts of scats that accumulate in a single location (several latrines yielded over 2kg each) (Sprent *et al.* 2006), suggest that latrines are the result of animals deliberately over-marking existing scats.

Possible functions of latrines in the social behaviour of echidnas

For many species, the distribution of scent marks, including latrines, are generally clustered in the outer regions of the territory or towards its centre (Roberts and Lowen 1997). Scent mark frequency may be inversely related to distance from the territory core and intruders advancing into the territory would be more likely to encounter marks and be made aware of ownership. Marks may also be placed in high densities where intrusion pressure is likely to be highest in areas of home range overlap. However, 90% kernel home ranges (as distinct from core home ranges) were generally much larger than the latrine survey area (up to 176ha (Nicol *et al.* 2011)). Combined with extensive overlap of 90% home ranges it was not possible to determine if latrines were located in the core or outer regions of echidna home ranges. As a result the distribution of latrines was examined in relation to core areas only.

If latrines function to defend use of resources located within core areas such as shelters, which are used throughout the year, then the highly heterogeneous landscape present at the study site may make it more profitable for echidnas to mark frequently in more valuable areas and not defend areas of marginal value (McLoughlin *et al.* 2000). If this is the case, then the higher incidence of latrines in scrub patches suggests that scrub may be of higher value for echidnas.

Surprisingly, in areas of high echidna density, as indicated by the number of overlapping core areas, there was no corresponding peak in the number of latrines, instead most latrines were located in areas of intermediate core area overlap. If high rates of scat deposition are a product of high occupancy by many individuals, then maximal latrine numbers would be expected in areas of maximal echidna density. However there was a peak in latrine numbers in areas of intermediate density. This suggests that latrines may function in regulating the spatial distribution of echidnas. At medium population densities latrines are present more frequently than at low and high densities. The degree of territoriality exhibited by individuals may be measured by home range overlap (Nemtzov 1997), and at low and high densities territorial defence may be abandoned due to the benefits of maintaining an exclusive area being outweighed by the energetic costs of defending it (Jarman 1979; Lockie 1966; Magurran and Seghers 1991; McLoughlin *et al.* 2000; Prieto and Ryan 1978). Although this study is based on a small area within a single population, the patterns of latrine numbers and animal density suggest that latrines may be used by echidnas to maintain their spatial organisation at medium densities. For example territoriality in brown bears *Ursus arctos*, as evidenced by low rates of home range overlap is maximal in areas of intermediate productivity and intermediate density. In areas of high productivity and population density, and low productivity and population density, territoriality appears to be abandoned and overlap of home ranges is extensive (McLoughlin *et al.* 2000). For echidnas, there may be no benefit of maintaining latrine sites if no other animals live close by, and in areas where there are high numbers of animals, latrine use and territorial behaviour may be abandoned due to the high energetic costs of needing to frequently over mark scats of other animals.

Another possible function of echidna latrines is as centres of information exchange. The large home ranges held by echidnas (male home range area 130 $\pm$ 236 ha, core area 42.6$\pm$ 15.9 ha, female home range area 50.1 $\pm$ 22 ha, core area 15.9 $\pm$ 7.5 ha, (Nicol *et al.* 2011) may result in infrequent direct encounters outside of the mating season. However, latrines in the overlapping portions of core areas could provide information on the reproductive and/or social status of male and female echidnas. For example, latrines function to transfer information from female to male bushbuck *Tragelaphus scriptus* (Wronski *et al.* 2006), and female European badgers *Meles meles* use latrines to exchange information about their reproductive status (Roper *et al.* 1993).

However, in Tasmanian echidnas, latrines are extremely unlikely to convey information from a female about her reproductive status. There would be limited opportunity for female latrine use during the reproductive season as female echidnas are normally still hibernating when mating occurs (Morrow and Nicol 2009). At Lovely Banks, mating is finished by early September (Morrow *et al.* 2009) while latrines were continually used between August and February (Sprent *et al.* 2006). Furthermore latrine use is not restricted to females as both males and females have been observed at latrines.

An alternative function of latrines in communication between echidnas is scent matching, whereby latrines provide an olfactory association between the predominant scent mark and a dominant individual (Begg *et al.* 2003), with dominant animals marking more than subordinates, and removing or replacing the marks of others. Whether dominance hierarchies exist within echidna populations is not known, but intense competition between males occurs during the mating season, where multiple males attempt to mate with the same female (Morrow *et al.* 2009; Rismiller and McKelvey 2000; Rismiller and Seymour 1991). If males use latrines to communicate dominance over other males around hibernating females before mating commences, then a seasonal peak in latrine use would be expected, and areas of higher latrine density should be associated with the locations of hibernating females. However, latrines are used throughout the active, non-hibernating months of the year, with no clear seasonal peak (Sprent *et al.* 2006). No mating groups were recorded within areas of high latrine density over the previous five years (pers. obs., G. Morrow, S. Nicol and N. Andersen) and latrines did not appear to correspond with the location of female hibernacula. Overall, the evidence suggests that latrines do not have a function related to mating, although they could possibly have some role in signalling the social status of males.

If the function of latrines is not closely related to mating, then the most likely alternative is that they provide a medium for transferring information about resource use, and are used by females to maintain access to resources within their home range. The size of the home ranges of female echidnas are influenced by the proportion of woodland habitat within them: smaller home ranges have a larger proportion of woodland than larger home ranges (Chapter 4). The higher densities of latrines in scrub woodland, and the higher frequency of latrines in areas of intermediate rather than high echidna density, suggest that through signalling her presence in high quality areas, a female echidna may

exclude other females. Thus resources are not depleted as quickly and she can access sufficient resources within a smaller area. In turn, male echidnas could use information from latrines to maintain their home ranges to maximise access to females.

Future work

There are still a number of questions about the function of latrines in echidna spatial ecology that remain unanswered. An important first step would be to identify which individuals are using specific latrines, and their pattern of usage. This could be done by using camera traps if echidnas have a unique visible tag, by use of a remote microchip reader that records all visits by micro chipped animals, or analysis of faeces for DNA. This would allow investigation of the relationship between location of latrines and the home ranges of the echidnas using them. Patterns of latrine use by males and females could then be used to determine if latrines are used to exclude other animals for home ranges or core areas or otherwise mediate spatiotemporal separation of neighbours, while patterns of use by males might reveal the existence of dominance hierarchies.

Chapter 6

General Discussion

General Discussion

Environments at polar and temperate latitudes are characterised by regular, predictable variations in day length, temperature and productivity throughout the year. Seasonal rhythms of activity, body mass and reproduction are common features of species inhabiting environments with distinct seasons, and have evolved in response to variable environmental productivity. Localised variations in habitat productivity also play a major role in the ecology and energetics of animals, influencing their diet, distribution and spatial organisation.

Echidnas throughout Australia show a strong seasonal cycle, with greatly reduced foraging activity and bouts of torpor in early winter (Nicol and Andersen 1996; Nicol and Andersen 2003). In Tasmania, the most southerly part of the echidna's range, this seasonality is at its greatest, and Tasmanian echidnas may hibernate for up to 8 months of the year (Nicol and Andersen 2007a). Both sexes show a large seasonal variation in body mass of approximately 30% associated with this seasonal cycle of activity and hibernation (see Appendix to Chapter 2), with body mass reaching its maximum prior to entry into hibernation. Females are at their minimum mass at the time they first leave the nursery burrow, while minimum mass for males is reached at the end of the reproductive period (Nicol and Andersen 2007a). The climate of Tasmania is not extreme – at the Southern Midlands field site, summers are warm (mean maximum 23.9°C) and winters mild (mean minimum 2.0°C) and rainfall is spread throughout the year (http://www.bom.gov.au/climate/ averages/tables/cw_094201.shtml). Hibernation in echidnas is not driven by low temperatures; animals enter hibernation in late summer when temperatures are still relatively warm, and exit from hibernation in mid winter (Morrow *et al.* 2009; Nicol and Andersen 1996; Nicol and Andersen 2002). So, if it is not directly driven by temperature, are there dietary or habitat factors that account for this extreme seasonal behaviour, which is not seen in any other similar sized Tasmanian mammal? The overall aims of this thesis were to investigate aspects of echidna seasonality, including the role of the lipostatic hormone leptin in the annual cycle of body mass and dietary and habitat effects that might be important energetically.

In the first part of this study, I investigated aspects of the physiological regulation of echidna seasonality. Specifically, I investigated the role of the hormone leptin in the annual cycle of hibernation and reproduction, with its attendant annual cycle of body mass. In eutherian mammals the peptide hormone leptin has a major role in the regulation of body mass by acting as feedback controller of adiposity (Ahima 2008). Leptin is synthesised and secreted primarily by adipose tissue and an increase in adiposity in most species, including hibernators, is normally associated with an increase in circulating leptin concentrations (Sahu 2004). High leptin levels inhibit feeding, and stimulate metabolism, thus regulating the amount of adipose tissue. I hypothesised that echidnas would also show this positive relationship between circulating leptin concentrations and adiposity, but like other hibernating mammals (Asikainen *et al.* 2004; Florant *et al.* 2004; Król *et al.* 2006; Kronfeld-Schor *et al.* 2000; Rousseau *et al.* 2002), a change in the relationship would occur during the period of prehibernatory fattening to prevent feedback inhibition of feeding during seasonal fattening. However, whilst leptin was present in the adipose tissue and plasma of echidnas, the expected positive relationship between circulating leptin concentrations and relative adiposity was not observed at any time of the year; instead there was a weak negative relationship. There were significant seasonal effects however - leptin was highest during hibernation and in females during the reproductive period, and lowest in post-reproductive males. The weak negative relationship suggests that leptin, although produced in echidna adipose tissue, is not functioning in the expected direct adipostatic fashion, and in this respect leptin in the echidna seems to follow the same pattern as in reptiles (Spanovich *et al.* 2006) and free ranging birds (Kordonowy *et al.* 2010; Quillfeldt *et al.* 2009).

While direct feedback control of adiposity is an important function of leptin in eutherian mammals, the more ancient role seems to be to suppress feeding (Crespi and Denver 2006). Exogenous leptin results in a reduction of food intake in a range of ectothermic and endothermic vertebrates (fish (Murashita *et al.* 2008) amphibians (Crespi and Denver 2006), reptiles (Niewiarowski *et al.* 2000), birds (Lõhmus *et al.* 2003) and mammals (Halaas *et al.* 1995; Hope *et al.* 1999)), and low circulating leptin concentrations coincide with periods of intensive foraging in mammals (Concannon *et al.* 2001; Li and Wang 2007). In the echidna, leptin is highest during those periods when feeding is minimal and lowest during periods of maximal foraging. In this way, leptin in

the echidna appears to have a role in regulating energy metabolism, but leptin release must be under the control of a factor, presumably dependent on day length and thus most probably melatonin (Alonso-Vale *et al.* 2005; Scherbarth and Steinlechner 2010). Melatonin production from the pineal gland is inversely proportional to day length, and in hamsters is the transducer in photoperiodic control of seasonal changes of adiposity (Bartness and Wade 1985). Additionally, in seasonally breeding Siberian hamsters, it appears that that hypothalamic responses to leptin are regulated primarily by photoperiod, rather than seasonal changes in fat reserves, sex steroids, or leptin concentrations (Rousseau *et al.* 2002). Therefore it seems likely that photoperiod may regulate leptin activity in echidnas.

The results of my study of leptin in echidnas are significant, not just for understanding the control of energy metabolism in the echidnas, but also because they provide information relevant to the evolutionary history of adiposity regulation. In this respect, echidna physiology appears to be closer to that of reptiles and birds than to eutherian mammals. The paucity of data from marsupials means that it is not clear when in the evolution of mammals the adiposity signalling function of leptin became established, but I speculate that the evolution of this role of leptin may be associated with the development of brown adipose tissue, which, although identified in marsupials (Jastroch *et al.* 2008), reaches its maximum expression in eutherian mammals (Hayward and Lisson 1992).

I also investigated the role of specific environmental factors in echidna seasonality and behaviour. While day length, signalled through melatonin, is the likely proximate stimulus for seasonal changes in food intake and energy expenditure (Alonso-Vale *et al.* 2005; Scherbarth and Steinlechner 2010), the seasonal cycle of mass could be a function of variation in foraging effort, varying food availability, or through echidnas changing their feeding preferences. Echidnas could, for example, concentrate on energy rich prey during prehibernatory fattening as observed in jumping mice (*Zapus princeps*) (Cranford 1978), fat tailed dwarf lemur (*Cheirogaleus medius)* (Fietz and Ganzhorn 1999) and edible dormouse (*Glis glis)* (Fietz et al. 2005).

Scat analysis indicated that diet consisted almost exclusively of ants and the larvae of pasture cockchafer beetles. Although the number of scats analysed was relatively low compared to other studies, there was no indication that termites, a major dietary

component of echidnas living in other areas, were consumed. This relatively small sample size was one of the limitations of this aspect of the study. Scats could not always be collected due to animals being inaccessible or not producing a sample. As a result, there may be some seasonal variation in the consumption of prey species that was not apparent. There was no significant seasonal variation of ant prey consumed, but consumption of cockchafer larvae was highly seasonal. This seasonality appears to be a simple function of availability of larvae, which is driven by their lifecycle. The black headed cockchafer (*Aphodius tasmaniae* and *A. pseudotasmaniae*) and yellow headed cockchafer (*Scitala sericans, Seicesthis nigra* and *Seicesthis nigrolineata*), have a 12 month life cycle, and during the prehibernation period in summer have developed into pupae and adult beetles (McQuillan *et al.* 2007), forms that are not consumed by echidnas. The red headed cockchafer (*Adoryphorus couloni*) which also occurs at the site has a two-year lifecycle, so although the other species show a significant reduction in availability in summer, some cockchafer larvae will always be available. Fig. 1 shows the mean monthly contribution of pasture cockchafer larvae to echidna diet. The months of lowest contribution coincide with the period of prehibernatory fattening in the echidna. It is also noteworthy that two scats, from two different individuals, consisting entirely of the large underground larvae of the lepidopteran *Oxycanus antipoda* were collected during this period. The availability of ants, the principal food source of echidnas at my field site, could not be readily determined, as the standard techniques for measuring ant abundance, such as food baits and pitfall traps, do not really give an indication of their availability to echidnas, which do not feed on surface ants. Without a measure of absolute abundance and availability however, it was not possible to draw any firm conclusions about diet selection and whether echidnas were targeting different prey types at key periods of the year. It would have been particularly interesting to determine if pre-hibernatory fattening was achieved through selection for different prey types during this period, and if hibernation was driven by a seasonal reduction in the availability of prey items.

The results from this study and previous studies allow me to make some interesting generalisations about the diet of Australian echidnas (there being no information on New Guinea short-beaked echidnas). Three main class groups of invertebrates make up the overwhelming bulk of the echidna diet: ants, termites and

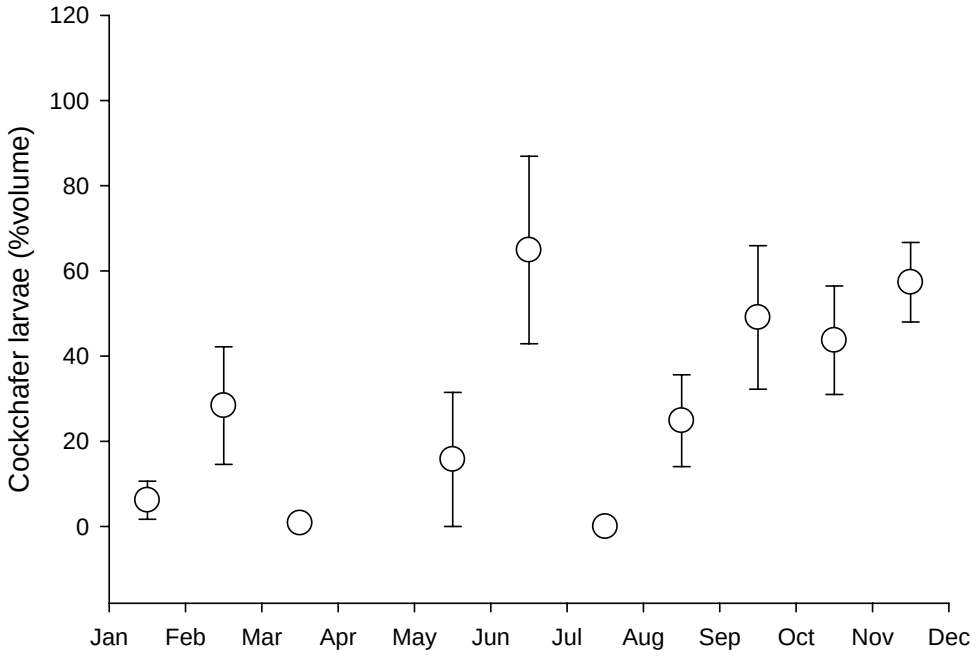

Fig. 1. The mean monthly contribution (% volume) of the larvae of the pasture cockchafer beetle to total diet of Tasmanian short beaked echidnas (*Tachyglossus aculeatus*). Error bars show standard error of the mean.

cockchafer larvae. Table 1 summarises the relative contribution of ants and termites to the diet of echidnas across their range in Australia, and whether larvae of cockchafer beetles are consumed. In arid areas where mound building termites are abundant, termites form a significant proportion of the diet of echidnas. However in more temperate regions, termites are significantly less abundant (Abensperg-Traun 1994) and in these areas ants make up a greater proportion of the diet, and beetle larvae become important. Australian lizards show a similar decrease in specialisation on termites in mesic temperate regions compared with arid environments (Abensperg-Traun 1994). Griffiths (1989) and Abensperg-Traun (1988) suggest that in arid areas, this preference for termites by echidnas is possibly due to their higher water content (up to 80%) compared to ants (64%). Alternatively, the higher consumption of termites may be due to preferential consumption where they are available due to their large colony size and lower amounts of indigestible chitinous exoskeleton, making them a better quality food source than ants (McNab 1984). In all areas where termites are abundant, they make up a large proportion of diet and the echidna is wholly myrmecophagous, consuming a diet of only ants and termites. However, the lower nutritional value of ants may mean that in areas where

Table 1: Summary of the patterns of ant, termite and beetle larvae consumption by short-beaked echidnas within different climatic zones across Australia. The number of plus signs reflects the relative importance of prey items in diet. If diet consisted of less than 4% of total diet then contribution is recorded as trace. A minus sign denotes no termites or beetle larvae were consumed. N.R denotes information on consumption was not reported.

Location	Climate	Ants	Termites	beetle larvae	Source
Kakadu Nat. Park. NT	Tropical	+++	+++	trace	(Griffiths *et al.* 1990)
Mileura district, WA	Arid	++	+++	N.R.	(Griffiths 1978)
Tibooburra, NSW	Arid	+++	++	-	(Lawes 2009)
Cunnamulla, Qld	Arid	+	++++	N.R.	(Griffiths 1978)
Central Australia, NT	Arid	++	+++	N.R	(Griffiths 1978)
Kellerberrin, WA	Semi-arid	++	+++	N.R	(Abensperg-Traun and De Boer 1992; Abensperg-Traun 1988)
Grenfell-Forbes district, NSW	Temperate	+++	++	N.R	(Griffiths 1978)
Kangaroo Island, SA	Temperate	+++	++	N.R	(Griffiths 1978)
Canberra, ACT	Temperate	+++	++	N.R	(Griffiths 1978)
New England, NSW	Temperate	++++	+	+++	(Smith *et al.* 1989)
Eucumbene district, NSW	Temperate	+++++	trace	N.R	(Griffiths 1978)
Strathbogie Ranges, Vic	Temperate	+++++	trace	+++	(Harrison 1997)
NE Tasmania	Temperate	+++++	-	+?	(Griffiths 1978; Spencer and Richards 2009)#
Melton Mowbray, Tas	Temperate	+++++	-	+++	Chapter 3

No quantitative data provided. Only presence of cockchafer larvae in scat and observation of echidnas feeding on larvae recorded.

termites are unavailable, echidnas must supplement their diet with other nutritional non-ant prey. The consumption of large proportions of pasture cockchafers in Tasmania (Chapter 3) is consistent with other populations of echidnas in temperate areas where termites are uncommon or absent. Across their range, echidnas appear to supplement their diet with better quality items than ants, which in northern areas are termites but in temperate, southern areas are the larvae of beetles. This significance of this essentially tripartite diet has not been noted in previous studies of the dietary ecology of echidnas.

Although the morphology of its feeding apparatus suggests that it is at the extreme end of specialisation on ants and termites (Reiss 2001) and would be expected to exclusively feed on ants and termites, the echidna is not restricted to a myrmecophagous diet, although ants appear to be an essential dietary item (Chapter 3). This specialisation may be relatively recent. Phillips *et al.* (2009) argue that echidnas have evolved from a platypus like ancestor which would have fed on benthic invertebrates, and three extinctspecies of echidna were probably not myrmecophagous; fossil records indicate that they consumed large insects, larvae of beetles and moths (Griffiths *et al.* 1991; Musser 2006). Although the long-beaked echidnas have similar adaptations of the feeding apparatus to short-beaked echidnas (Griffiths 1978; Reiss 2001), the eastern long-beaked echidna of New Guinea (*Zaglossus bartoni*) feeds on earthworms and other soil invertebrates (Griffiths 1978; Milewski *et al.* 1994; M. Opiang, pers. comm.). As has been demonstrated in the nine banded armadillo (*Dasypus nivemcinctus*), anatomical specialisation does not preclude dietary variation (Smith and Redford 1990) and, in the absence of termites, the short-beaked echidna is able to supplement an ant diet with other soft-bodied soil invertebrates.

Chapter 3 illustrated strongly the different information about diet provided by stable isotope analysis (SIA) and scat analysis. The lack of apparent seasonal variation in diet from SIA, despite significant variation in the consumption of cockchafer larvae evident from scat analysis, highlights the difference in the type of information provided by these types of analysis. Stable isotope analysis of body tissues reflect the assimilation of C and N over a period that is determined by the rate of turnover of the particular tissue sampled, unlike the instant "snapshot" of diet obtained from scat analysis. This is one of the likely explanations of the lack of seasonal variation in diet apparent from isotopic analysis of

blood. The half-life of red cells in euthermic echidnas is likely to be similar to other mammals, i.e. about 15 - 40 days (Brock 1960) but it is likely to increase significantly in hibernating echidnas (Andersen *et al.* 2000). In golden hamsters red-cell half life increases from 15 days in euthermic animals to 160 days during hibernation (Brock 1960). Thus, depending on when the sample is taken, $\delta^{15}N$ and $\delta^{13}C$ values of echidna blood could reflect assimilation of a diet consumed weeks or several months previously. A great strength of SIA is that it can be used to infer foraging locations, whereas scat analysis only provides information on the actual diet consumed – or at least the identifiable remnants of the undigested portion of that diet. The most surprising feature of the SIA was that it did not indicate the presence of cockchafer larvae in the diet although scat analysis showed them to be a major dietary component. However the very distinct isotopic signature of the larvae may be due to the fact that only larvae from pasture were analysed, and larvae from woodland probably have a significantly different isotopic composition.

With the exception of cockchafer larvae, comparisons of isotope ratios in the blood with those in dietary items indicate that echidnas foraged within the predominant vegetation type of their home range. This demonstrates the importance of determining actual composition of diet through methods such as scat analysis, in addition to determining isotopic signatures of potential prey items, when using SIA to examine foraging ecology. The difference in the isotopic signatures of ants in different habitats has been previously observed; their trophic position changing with habitat (Gibb and Cunningham 2011). The trophic 'promiscuity' of omnivorous insects is widespread (Hunter 2009), but few studies have examined the effect of this flexible diet strategy on the stable isotopic signature of the consumers of these species. Without the prior knowledge that echidnas are consuming the same prey items throughout the site, $\delta^{15}N$ of blood from echidnas inhabiting different habitat types would suggest that the trophic level at which echidnas are feeding differs. This could have lead to the incorrect conclusion that diet of echidnas varies greatly between different habitats, and demonstrates the value of using more than one method of diet analysis, particularly on insectivorous species.

The diet of echidnas will depend on the local availability of items they are capable of eating. As noted above it was not practicable to estimate seasonal variation in ant availability during this project, but it is possible to estimate overall seasonal variation in ecosystem productivity. Fig 2 shows estimated mean monthly C_3 and C_4 pasture productivity at the Lovely Banks field site, calculated using the pasture simulation model EcoMod (Johnson *et al.* 2008). This model uses climatic data from the site, including temperature, day length, rainfall and evapotranspiration records for the last 15 years. Superimposed on this are curves showing the proportion of male and female echidnas that are active (data from Nicol and Andersen (2002)), the occurrence of mating groups (data from Morrow *et al.*(2009)), and the growth of young (data from Nicol and Andersen (2007a)). An increase in plant productivity results in a corresponding increase in the abundance of ant species (Kaspari 2001; Siemann 1998), and C_3 and C_4 productivity calculated in this model can be used as a proxy for abundance of potential prey items of echidnas. The only C_4 plant at the field site is kangaroo grass (*Themeda triandra*) (M. Hovenden, pers. comm.) which occurs in areas of native pasture, so the overall ecosystem productivity will be better represented by the C_3 curve. During the period January – April (prehibernation and early hibernation), overall productivity is quite unreliable, and cockchafer intake is at its lowest (Fig. 1). Ant availability is likely to follow ecosystem productivity – generally an increase in productivity results in a corresponding increase in the abundance of faunal species (Kaspari 2001; Siemann 1998), and consequently C_3 and C_4 productivity can be used as a proxy for abundance of potential prey items of echidnas.

Ecosystem productivity and thus presumably food availability are at their lowest during the echidna mating period (grey bars in Fig. 2). During winter ants reduce their foraging activity, and the most nutritious ant food items for echidnas, ant pupae and larvae (Redford and Dorea 1984), are greatly reduced or absent (Thomas 2003). Echidna foraging is limited and echidnas must draw on fat stores, and their plasma leptin is high. The timing of reproduction means that maximum growth of young, which is being supported by the lactating mother, coincides with high and reliable ecosystem productivity, and thus maximum food availability. This pattern is seen in most seasonally breeding mammals (Malpaux 2006), including Columbian ground squirrels (*Urocitellus columbianus*) which synchronise their reproductive period with upcoming peaks in

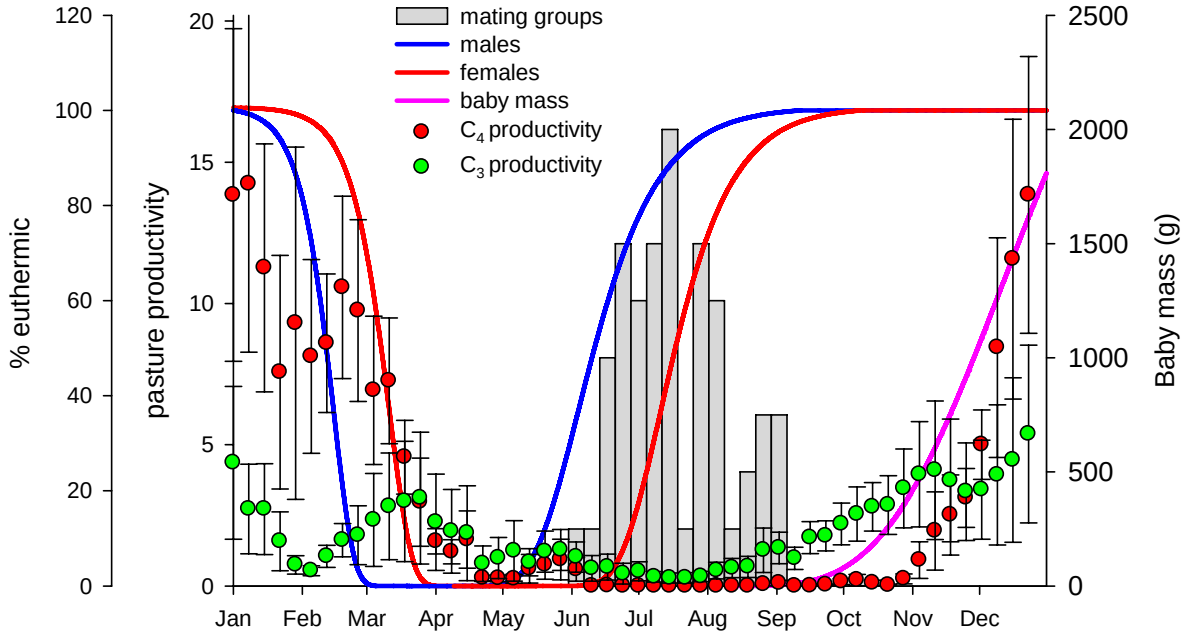

Fig. 2. Model of mean monthly C_3 and C_4 productivity for pasture (Kg DM.ha^{-1}.day^{-1}) at Lovely Banks. Most vegetation at the site is C_3 (most grasses, and trees), but there are also extensive patches of C_4 grass (kangaroo grass *Themeda triandra*). During hot weather C_4 grasses have high rates of growth. The number of male and female echidnas that are euthermic (i.e. not hibernating), frequency of mating groups and growth of young is also included to demonstrate how the timing of these aspects of the annual cycle of echidnas corresponds with primary productivity. Error bars denote standard error of the mean. C_3 and C_4 productivity modelling by Mike Perrring and Mark Hovenden (School of Plant Science, University of Tasmania). Data on male and female hibernation from Nicol and Andersen (2002), growth of young from Nicol and Andersen (2002), and occurrence of mating groups data from Morrow (2009).

vegetation abundance (Lane *et al.* 2011). The 140 day lactation period of Tasmanian echidnas (Morrow *et al.* 2009) means for this to happen, mating must occur in early winter. If Tasmanian echidnas had the same lactation period as Kangaroo Island and West Australian echidnas (about 207 days, (Morrow *et al.* 2009), mating would have to occur in May for weaning to occur at the optimum time. Thus there must be quite strong selective pressure to synchronise reproduction and lactation with ecosystem productivity throughout Australia.

Given the large seasonal variation in energy expenditure, and thus of food requirements of male and female echidnas, it might be expected that males and females would forage in different types of habitat at different times of the year as observed in giraffes (*Giraffa camelopardalis tippelskirchi)* (Ginnett and Demment 1997) and squirrel monkeys (*Saimiri oerstedi*) (Boinski 1983). During lactation, female echidnas may forage in areas that offer high energetic returns and for males foraging in these areas would be expected during the mating period as males that are able to meet their energetic needs in shorter time could increase fitness by spending more time vying for mating opportunities. However the analysis of the stable isotope data showed that despite the very significant effect of habitat on the red cell stable isotopes, there was no seasonal variation, implying that echidnas foraged in the same habitat type throughout the year. Whilst the lack of seasonal variation may be due to the relatively small number of samples analysed, this is consistent with the data obtained from radio tracking (Nicol et al 2011), and shows that all the resources required by echidnas are available in their home range throughout the year. However, the study site is a highly heterogeneous mix of pasture interspersed with patches of woodland, and total productivity and resource availability can be expected to vary between habitat types. In Chapter 4, I examined the influence of habitat type on echidna home range. Strong relationships between habitat quality and home range size in other species are commonly linked with food availability and productivity (e.g. (Broughton and Dickman 1991; Herfindal *et al.* 2005; McLoughlin *et al.* 2000; Tufto *et al.* 1996)). The size of home ranges of female echidnas at Lovely Banks was also influenced by the availability and quality of resources within different habitat types, although home range sizes of male echidnas were not. Females had significantly smaller home ranges than males, and the size of female home ranges was highly dependent on the

proportion of woodland habitat within them. Home ranges of females inhabiting woodland were smaller than those in pasture, suggesting that woodland provides better quality habitat. Though I could not draw a firm conclusion about food availability in different habitats, woodland provides a higher degree of structural complexity than pasture, which is likely to influence the distribution of ant nests and cockchafer larvae. Furthermore, the limited data available on the ant communities at Lovely Banks indicates that prey availability in woodland may be more predictable throughout the year as it is less influenced by seasonal variation than pasture (Yu Ka Ying 2010), further suggesting that woodland is higher quality habitat than pasture.

Metabolic rates of male and female echidnas are similar (Nicol and Andersen 2007a) and there is no difference in diet (Chapter 3), so it appears that home ranges of male echidnas are larger than is required to meet their metabolic requirements. Males are probably scaling their home ranges not on food or shelter availability, but, as in the case of other solitary species with a promiscuous mating system (Clutton-Brock 1989; Sandell 1989), they are maximising their access to females. The selective pressure on male echidnas is to maximise chances of reproductive success by mating with as many females as possible, and accordingly, their home ranges overlap with many female home ranges and are much larger. For female echidnas there would be strong selective pressure to secure sufficient resources within as small an area as possible for successful rearing of young, as well as to regain energy stores for hibernation.

The size of female echidna home ranges may be driven more by the availability of sheltering opportunities than food resources. Although echidnas avoid the drop in primary productivity over autumn and winter by hibernating, they still need access to essential non-food resources, in particular suitable shelter. Hibernacula and nursery burrows are distributed throughout the home ranges of females and they forage across their home range. Consequently, female home ranges must be scaled to encompass all these requirements across the year. There is no difference in the size of home range of echidnas during hibernation than during the active period (Nicol *et al.* 2011), and echidnas frequently re-use the same shelter, indicating that shelters may be a limiting resource, as has been argued by Wilkinson *et al.* (1998). Home ranges of echidnas, in some circumstances at least, may be determined more by shelter availability than, food availability, as has been suggested by Smith *et al.* (1989). The exceptionally large home

ranges observed in American martens (*Martes Americana*) did not result from diminished prey numbers size (Smith and Schaefer 2002), and adjustment in the home range size of roe deer (*Capreolus capreolus*) was only partially in response to decreasing food supply (Tufto et al. 1996). These results all suggest that mechanisms other than purely metabolic requirements many influence home range size.

Higher quality, more productive habitat will have greater food resources available, as well as other resources such as shelter, and good habitat should be able to support a higher density of animals (Zalewski and Jędrzejewski 2006). But how is the distribution of animals in the landscape regulated? Even at high densities, solitary species like the echidna, may have limited opportunities for direct contact between individuals. Many solitary species, particularly carnivores, use latrines to communicate presence, reproductive status or dominance, and the final chapters of this thesis describe echidna latrines, large accumulations of echidna faeces at specific sites (Chapter 5a), and the relationship of these latrines to the local habitat and to known echidna home ranges (Chapter 5b). Scat deposition by echidnas occurred throughout the active season and there was no evidence of a peak in use corresponding to the reproductive season. This suggests that latrines much have a function other than signalling reproductive status (Begg *et al.* 2003; Ruibal *et al.* 2010). Areas of scrub had greater density of latrines than open pasture or thick bush, but surprisingly, high latrine density was not a reflection of higher animal density. The higher density of latrines in scrub may indicate that this habitat type is of greater value to echidnas than open pasture or thick bush. For example scent marking by freshwater otters (*Lutra lutra)* is primarily related to resource availability and marking behaviours are positively correlated with fish biomass (Prenda and Granadolorencio 1996). The smaller size of home ranges in woodland habitat suggests that it is higher quality than pasture (Chapter 4). Although analysis for home range did not split woodland habitat into the finer scaled categories of scrub and thick bush used to explore the spatial distributions of echidna latrines, the results of these two chapters both suggest that pasture habitat was lower quality. Overall, the pattern of latrine locations and echidna home ranges suggests that latrines may be used by echidnas to maintain their spatial organisation at medium densities.

Future work

To fully understand the effects and role of leptin in the annual cycle of echidnas more detailed study is required. Collection of a series of frequent samples from approximately 20 male and female echidnas, along with detailed information about their activity and physiological state, would eliminate the effect of having to group the data by date, which reduced the ability to determine how leptin interacted with physiology in the current study. By correlating leptin concentrations with detailed data on reproductive activity, including testosterone and progesterone concentrations, hibernation and foraging behaviour, the function of leptin in regulating physiology and behaviour could be explored. Experimental research on the effects of exogenously administered leptin on foraging and hibernation may provide further insight into the evolution of leptin function in monotremes and other mammals.

Isotopic mixing models enable the calculation of relative contribution of prey items to total diet, but are dependant on adequate isotopic information of prey items. For many species, the breadth of the diet prevents sampling of all prey species; however, as determined from scat analysis, the species diversity of prey consumed by echidnas at Lovely Banks is relatively low. With intensive searching, including overnight, it should be feasible to collect samples of each ant species from different habitats across the site. The use of red blood cells, which have a slower turnover than blood plasma, may have masked any seasonal variation in prey consumption. Using tissues with a more rapid turnover such as blood plasma, combined with a frequent sampling protocol, would give a more accurate indication of dietary change between seasons throughout the year. Simultaneous analysis of scats collected from individuals at the same time as blood sampling would provide highly detailed information about the spatial and temporal factors influencing the foraging ecology of the echidna, although scats may be produced by echidnas upon handling, it can not be relied on.

Home range is commonly viewed as the sum of an animal's movements and does not take into account the spatial distribution of resources that structure an animal's movements (Mitchell and Powell 2004). Thus home ranges estimated using traditional methods such as kernel analysis and MCP will consist of patches of resource rich habitat embedded within less valuable areas which are primarily only used for travel, or not at all. The concept of home range that is generally used in spatial research only indicates

where an animal was found most frequently at the time of sampling rather than an accurate reflection of use. This study has shown that attachment of GPS loggers to echidnas is easily accomplished, although the units themselves proved problematic. Recent, rapid development in spatial technology has meant that tracking fine scale movements of animals such as echidnas is becoming more feasible. New generation GPS loggers, packaged within considerably smaller units with extended battery life, have the ability to identify the actual area an individual uses within its more traditionally defined home range. Deployment of loggers at specific times of the year will differentiate the habitat areas that are important for different behavioural activities including foraging, hibernation and rearing of young, information fundamental to understanding how animals function within their environment.

GPS loggers could also be used to study latrine use, providing insight into the function of latrines in the spatial ecology of echidnas. DNA based techniques may also be a key to understanding their role. Species specific microsatellite and primer design has recently been completed for echidnas and this could be used to determine the sex and identity of individuals using latrines. Similarly, camera trapping of individuals at latrine sites would also provide information on the identity of individuals using latrines. Knowledge about the location of latrines used by known animals within a given individual's home range and their distribution in relation to male and female home ranges should help confirm the function of latrines in the spatial ecology of echidnas.

Conclusions

This study, based on a population of echidnas in the Southern Midlands of Tasmania, has produced results that are significant at a number of levels.

- It provides insights into the evolution of the function of leptin, and seasonal regulation of energy stores in a hibernating species. Monotremes secrete leptin into the blood, but like reptiles and birds, and unlike eutherian mammals, plasma leptin concentration does not appear to provide information on the state of adipose tissue stores. Leptin does appear to have a role in suppressing feeding and activity at appropriate stages of the annual cycle of hibernation and reproduction, which may reflect its ancestral role in vertebrates.

- The perception of the echidnas as a purely myrmecophagous species needs to be reassessed within its distribution throughout temperate parts of Australia. Echidnas at the study site ate two major categories of prey – ants and cockchafer larvae. Comparison with data from other parts of Australia reveals a pattern that suggests that echidnas require additional prey beyond ants. In areas where they are abundant, a large number of termites are consumed and as availability of termites drops the consumption of cockchafer larvae increases. In spite of their extreme morphological specialization for a myrmecophagous diet the echidna is able to supplement its diet with non-ant prey when termites are unavailable. This dietary flexibility probably accounts for their almost ubiquitous distribution throughout Australia.

- The relationship between habitat quality and home range size has been demonstrated in a myrmecophagous species for the first time. Like many other solitary species with a promiscuous mating system, echidnas have significant sex linked differences in the relationship between habitat quality and home range size. Home ranges of female echidnas are based on access to resources, whereas the home ranges of males are probably based on access to females. However the factors which drive the spatial distribution of females appear to deviate from that in other species. Although there may be some variation in food availability between habitat types, generally the prey of echidnas is widely distributed and abundant. Instead of being primarily driven by food availability, female organisation may be more strongly related to other resources possibly the availability of shelter.

- Echidna latrines have been described and their location in relation to the home ranges of individual echidnas determined. The solitary echidna may rarely directly encounter other individuals. Echidna latrines provide a likely method of communication between animals. Their relationship with the distribution of home ranges suggests they function in the maintenance of spatial organisation.

This thesis has provided some interesting insights into the influence of seasonal variation and habitat on the energetics, diet and spatial ecology of echidnas. It provides new

evidence of the place of monotremes in the evolution of energetic pathways between reptiles, birds and mammals, and queries the concept that the echidna is a purely myrmecophagous species. For the first time, the relationship between habitat quality and home range size has been demonstrated in a myrmecophagous species, and makes a first step towards understanding how echidnas maintain their spatial organisation.

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